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FGFR redundancy limits the efficacy of FGFR4-selective inhibitors in hepatocellular carcinoma

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This PDF file includes:
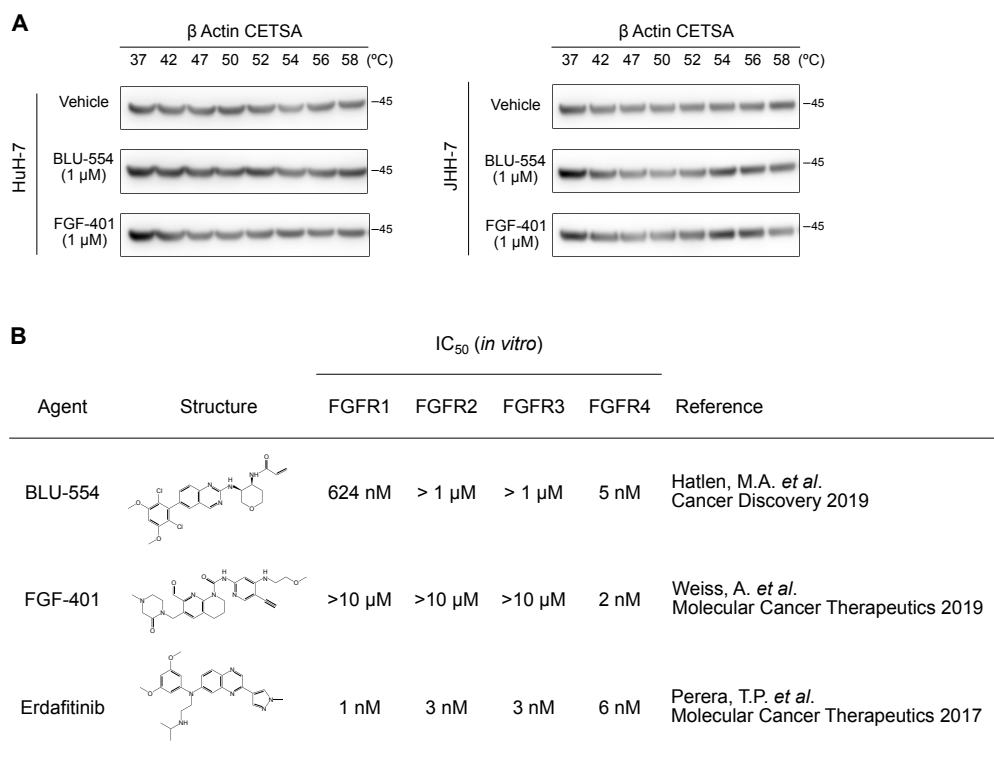
Figures S1 to S6
Tables S1 to S3

A

β Actin CETSA

	37 42 47 50 52 54 56 58 (ºC)
HuH-7	Vehicle ▭ —45
	BLU-554 (1 µM) ▭ —45
	FGF-401 (1 µM) ▭ —45

β Actin CETSA

	37 42 47 50 52 54 56 58 (ºC)
JHH-7	Vehicle ▭ —45
	BLU-554 (1 µM) ▭ —45
	FGF-401 (1 µM) ▭ —45

B

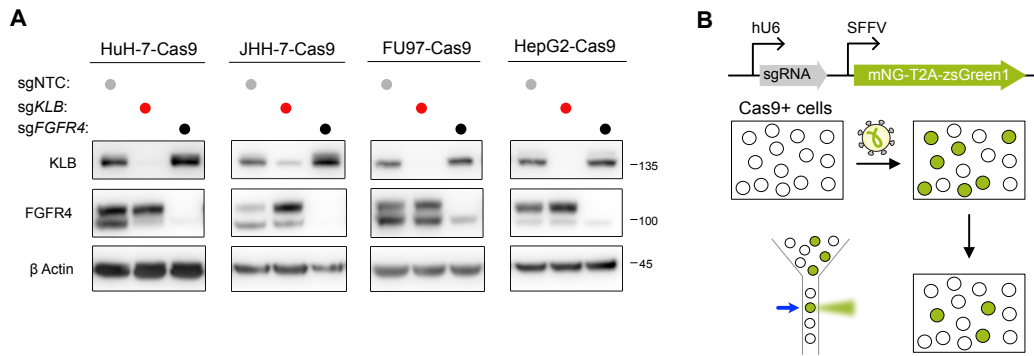| Agent	Structure	IC$_{50}$ (*in vitro*)				Reference
		FGFR1	FGFR2	FGFR3	FGFR4	
BLU-554		624 nM	> 1 µM	> 1 µM	5 nM	Hatlen, M.A. *et al*. Cancer Discovery 2019
FGF-401		>10 µM	>10 µM	>10 µM	2 nM	Weiss, A. *et al*. Molecular Cancer Therapeutics 2019
Erdafitinib		1 nM	3 nM	3 nM	6 nM	Perera, T.P. *et al*. Molecular Cancer Therapeutics 2017

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2 Fig. S1: CETSA loading control (β Actin) (A) and *in vitro* potency and selectivity of BLU-554,

3 FGF-401, and erdafitinib reported previously (B).

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Fig. S2: *In vitro* competitive cell growth assay to quantify the viability effects after CRISPR

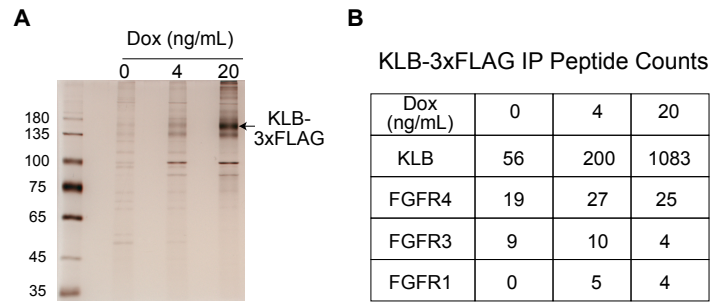**inactivation of *KLB* and *FGFR4*.** (a)Verification of *KLB* and *FGFR4* CRISPR knockout

efficiencies in indicated cell lines by western blotting. (b) Diagram of the *in vitro* competitive cell

growth assay. Cas9-transduced cells were infected with lentivirus expressing an sgRNA and a

fluorescent protein marker at a low multiplicity of infection (0.2-0.4). Flow cytometry was used

to monitor the percentages of transduced GFP+ cells over time.

8

A

Dox (ng/mL)

B

KLB-3xFLAG IP Peptide Counts

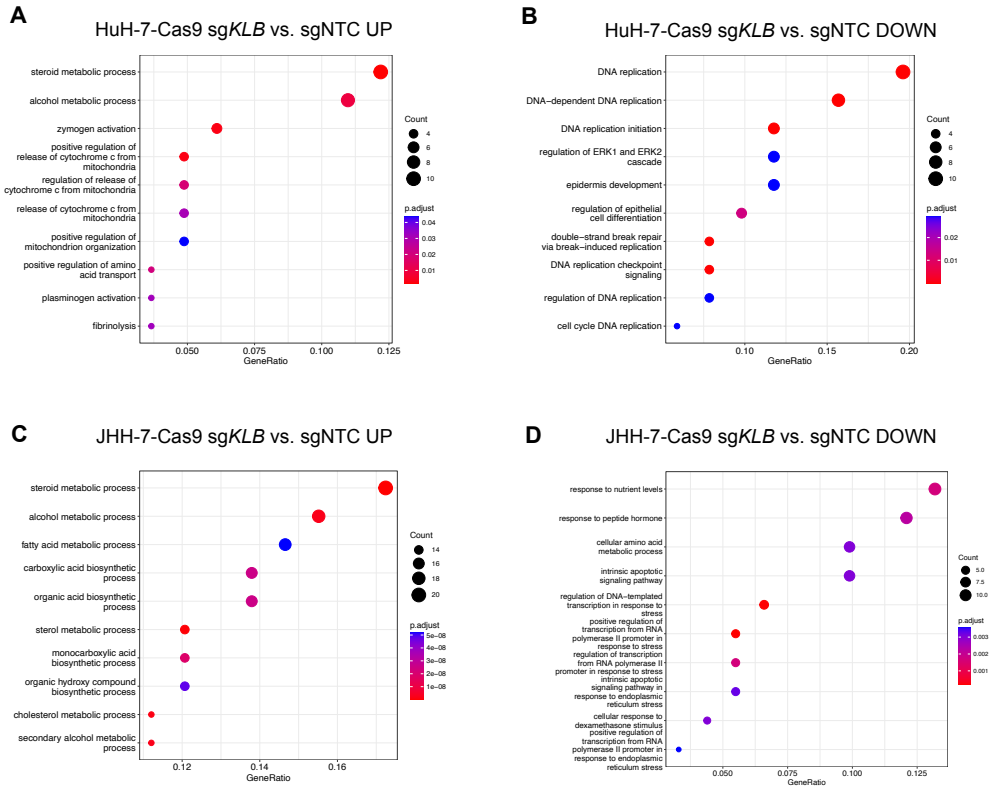| Dox (ng/mL)	0	4	20
KLB	56	200	1083
FGFR4	19	27	25
FGFR3	9	10	4
FGFR1	0	5	4

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2 **Fig. S3: IP-MS of KLB-3xFLAG complex purified from HuH-7 cells. (a)**Visualization of the

3 KLB-3xFLAG complex immunopurified from HuH-7 cells by SDS-PAGE and silver staining.

4 KLB-3xFLAG was expressed by the tetracycline-inducible (Tet-On) system. **(b)** Total peptide
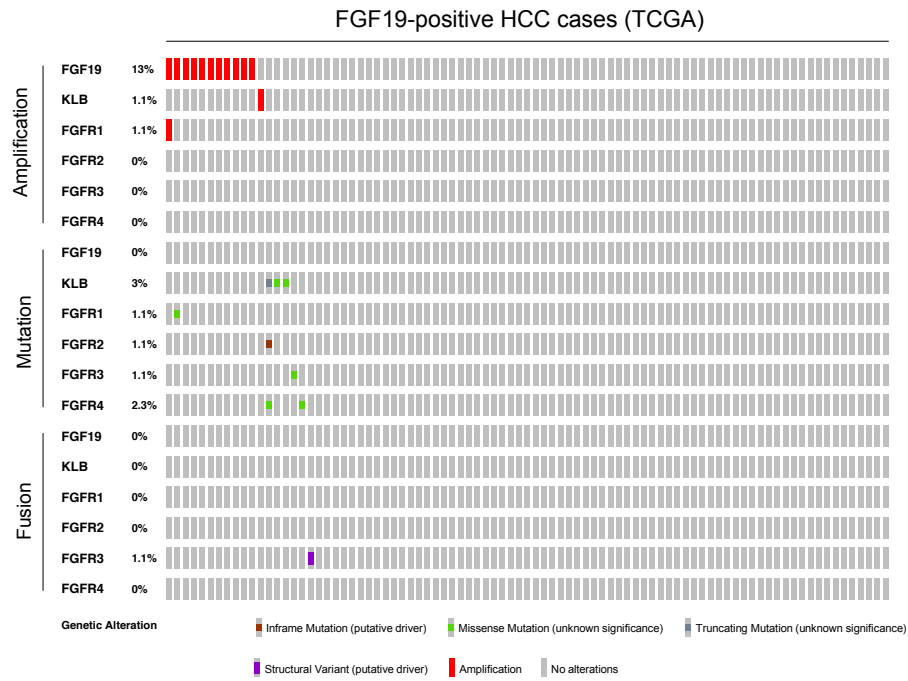
5 counts of indicated proteins identified in the KLB-3xFLAG complex by mass spectrometry.

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A HuH-7-Cas9 sg*KLB* vs. sgNTC UP

B HuH-7-Cas9 sg*KLB* vs. sgNTC DOWN

C JHH-7-Cas9 sg*KLB* vs. sgNTC UP
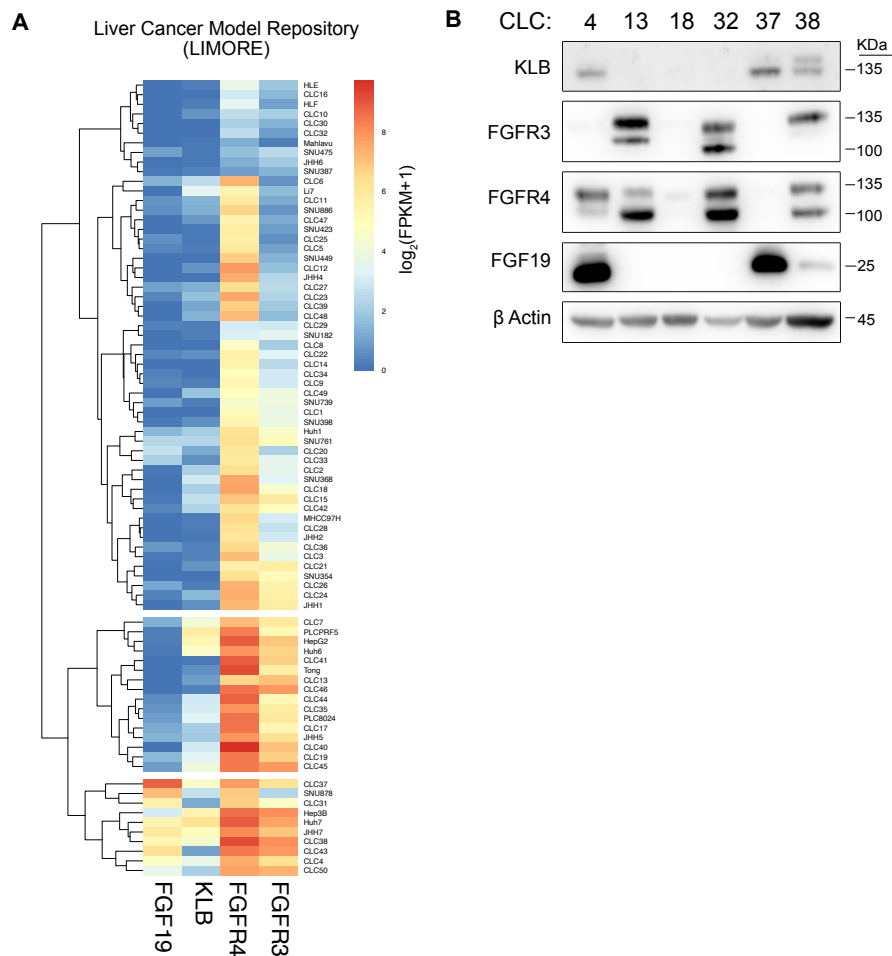
D JHH-7-Cas9 sg*KLB* vs. sgNTC DOWN

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2   **Fig. S4: Gene ontology enrichment analysis of differentially expressed genes in HuH-7-Cas9**

3   **and JHH-7-Cas9 cells after CRISPR inactivation of *KLB*.**

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Fig. S5: Analysis of genetic alterations of *FGF19*, *KLB*, *FGFR1/2/3/4* in *FGF19*-positive HCC cases in TCGA.

Fig. S6: Expression of FGF19, KLB, FGFR3, and FGFR4 of cell lines from the LIMORE collection. (a) Heatmap depicting mRNA levels of *FGF19*, *KLB*, *FGFR3*, and *FGFR4* in LIMORE cell lines. **(b)** Western blots showing proteins levels of FGF19, KLB, FGFR3, and FGFR4 in 6 cell lines from LIMORE.

1 **Supplementary Tables**

2 **Table S1:** Tukey's multiple comparison test (*P* values) for Fig. 5B.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
sgNTC vs. sgKLB	7.687	6.631 to 8.742	Yes	****	<0.000001
sgNTC vs. sgFGFR1	-0.4016	-1.457 to 0.654	No	ns	0.943019
sgNTC vs. sgFGFR3	3.268	2.213 to 4.324	Yes	****	<0.000001
sgNTC vs. sgFGFR4	5.835	4.779 to 6.89	Yes	****	<0.000001
sgNTC vs. sgFGFR1/3	3.723	2.668 to 4.779	Yes	****	<0.000001
sgNTC vs. sgFGFR1/4	5.208	4.152 to 6.263	Yes	****	<0.000001
sgNTC vs. sgFGFR3/4	9.246	8.19 to 10.3	Yes	****	<0.000001
sgNTC vs. sgFGFR1/3/4	9.394	8.339 to 10.45	Yes	****	<0.000001
sgKLB vs. sgFGFR1	-8.088	-9.144 to -7.033	Yes	****	<0.000001
sgKLB vs. sgFGFR3	-4.419	-5.474 to -3.363	Yes	****	<0.000001
sgKLB vs. sgFGFR4	-1.852	-2.908 to -0.7965	Yes	****	0.000028
sgKLB vs. sgFGFR1/3	-3.964	-5.019 to -2.908	Yes	****	<0.000001
sgKLB vs. sgFGFR1/4	-2.479	-3.535 to -1.423	Yes	****	<0.000001
sgKLB vs. sgFGFR3/4	1.559	0.5032 to 2.614	Yes	***	0.000543
sgKLB vs. sgFGFR1/3/4	1.707	0.6518 to 2.763	Yes	***	0.000122
sgFGFR1 vs. sgFGFR3	3.67	2.614 to 4.725	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR4	6.236	5.181 to 7.292	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR1/3	4.125	3.069 to 5.18	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR1/4	5.609	4.554 to 6.665	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR3/4	9.647	8.592 to 10.7	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR1/3/4	9.796	8.74 to 10.85	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR4	2.567	1.511 to 3.622	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR1/3	0.455	-0.6005 to 1.511	No	ns	0.890379
sgFGFR3 vs. sgFGFR1/4	1.94	0.8842 to 2.995	Yes	****	0.000011
sgFGFR3 vs. sgFGFR3/4	5.977	4.922 to 7.033	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR1/3/4	6.126	5.071 to 7.182	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/3	-2.112	-3.167 to -1.056	Yes	****	0.000002
sgFGFR4 vs. sgFGFR1/4	-0.627	-1.682 to 0.4286	No	ns	0.594428
sgFGFR4 vs. sgFGFR3/4	3.411	2.355 to 4.466	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/3/4	3.559	2.504 to 4.615	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR1/4	1.485	0.4292 to 2.54	Yes	**	0.001122
sgFGFR1/3 vs. sgFGFR3/4	5.522	4.467 to 6.578	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR1/3/4	5.671	4.616 to 6.727	Yes	****	<0.000001
sgFGFR1/4 vs. sgFGFR3/4	4.038	2.982 to 5.093	Yes	****	<0.000001
sgFGFR1/4 vs. sgFGFR1/3/4	4.186	3.131 to 5.242	Yes	****	<0.000001
sgFGFR3/4 vs. sgFGFR1/3/4	0.1487	-0.9069 to 1.204	No	ns	0.999936

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1 **Table S2:** Tukey's multiple comparison test (*P* values) for Fig. 5C.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
sgNTC vs. sgKLB	3.25	2.873 to 3.627	Yes	****	<0.000001
sgNTC vs. sgFGFR1	0.8636	0.4863 to 1.241	Yes	****	<0.000001
sgNTC vs. sgFGFR3	0.3964	0.01911 to 0.7738	Yes	*	0.032278
sgNTC vs. sgFGFR4	0.6048	0.2275 to 0.9822	Yes	****	0.000093
sgNTC vs. sgFGFR1/3	1.267	0.8898 to 1.645	Yes	****	<0.000001
sgNTC vs. sgFGFR1/4	1.104	0.7265 to 1.481	Yes	****	<0.000001
sgNTC vs. sgFGFR3/4	2.544	2.167 to 2.921	Yes	****	<0.000001
sgNTC vs. sgFGFR1/3/4	3.035	2.658 to 3.413	Yes	****	<0.000001
sgKLB vs. sgFGFR1	-2.386	-2.764 to -2.009	Yes	****	<0.000001
sgKLB vs. sgFGFR3	-2.853	-3.231 to -2.476	Yes	****	<0.000001
sgKLB vs. sgFGFR4	-2.645	-3.022 to -2.268	Yes	****	<0.000001
sgKLB vs. sgFGFR1/3	-1.983	-2.36 to -1.605	Yes	****	<0.000001
sgKLB vs. sgFGFR1/4	-2.146	-2.523 to -1.769	Yes	****	<0.000001
sgKLB vs. sgFGFR3/4	-0.7057	-1.083 to -0.3284	Yes	****	0.000004
sgKLB vs. sgFGFR1/3/4	-0.2144	-0.5918 to 0.1629	No	ns	0.665491
sgFGFR1 vs. sgFGFR3	-0.4672	-0.8445 to -0.08986	Yes	**	0.005373
sgFGFR1 vs. sgFGFR4	-0.2588	-0.6361 to 0.1185	No	ns	0.416431
sgFGFR1 vs. sgFGFR1/3	0.4035	0.02621 to 0.7809	Yes	*	0.027284
sgFGFR1 vs. sgFGFR1/4	0.2402	-0.1371 to 0.6175	No	ns	0.519345
sgFGFR1 vs. sgFGFR3/4	1.681	1.303 to 2.058	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR1/3/4	2.172	1.794 to 2.549	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR4	0.2084	-0.169 to 0.5857	No	ns	0.698656
sgFGFR3 vs. sgFGFR1/3	0.8707	0.4934 to 1.248	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR1/4	0.7074	0.33 to 1.085	Yes	****	0.000003
sgFGFR3 vs. sgFGFR3/4	2.148	1.77 to 2.525	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR1/3/4	2.639	2.262 to 3.016	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/3	0.6624	0.285 to 1.04	Yes	****	0.000015
sgFGFR4 vs. sgFGFR1/4	0.499	0.1217 to 0.8763	Yes	**	0.002228
sgFGFR4 vs. sgFGFR3/4	1.939	1.562 to 2.317	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/3/4	2.431	2.053 to 2.808	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR1/4	-0.1634	-0.5407 to 0.214	No	ns	0.897244
sgFGFR1/3 vs. sgFGFR3/4	1.277	0.8996 to 1.654	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR1/3/4	1.768	1.391 to 2.146	Yes	****	<0.000001
sgFGFR1/4 vs. sgFGFR3/4	1.44	1.063 to 1.818	Yes	****	<0.000001
sgFGFR1/4 vs. sgFGFR1/3/4	1.932	1.554 to 2.309	Yes	****	<0.000001
sgFGFR3/4 vs. sgFGFR1/3/4	0.4913	0.1139 to 0.8686	Yes	**	0.00277

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1 **Table S3:** Tukey's multiple comparison test (*P* values) for Fig. 6E.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
sgNTC vs. sgKLB	4.018	3.702 to 4.334	Yes	****	<0.000001
sgNTC vs. sgFGFR1	0.184	-0.1323 to 0.5003	No	ns	0.606452
sgNTC vs. sgFGFR3	0.474	0.1577 to 0.7903	Yes	***	0.000552
sgNTC vs. sgFGFR4	2.678	2.362 to 2.994	Yes	****	<0.000001
sgNTC vs. sgFGFR1/3	0.786	0.4697 to 1.102	Yes	****	<0.000001
sgNTC vs. sgFGFR1/4	2.406	2.09 to 2.722	Yes	****	<0.000001
sgNTC vs. sgFGFR3/4	3.854	3.538 to 4.17	Yes	****	<0.000001
sgNTC vs. sgFGFR1/3/4	3.564	3.248 to 3.88	Yes	****	<0.000001
sgKLB vs. sgFGFR1	-3.834	-4.15 to -3.518	Yes	****	<0.000001
sgKLB vs. sgFGFR3	-3.544	-3.86 to -3.228	Yes	****	<0.000001
sgKLB vs. sgFGFR4	-1.34	-1.656 to -1.024	Yes	****	<0.000001
sgKLB vs. sgFGFR1/3	-3.232	-3.548 to -2.916	Yes	****	<0.000001
sgKLB vs. sgFGFR1/4	-1.612	-1.928 to -1.296	Yes	****	<0.000001
sgKLB vs. sgFGFR3/4	-0.164	-0.4803 to 0.1523	No	ns	0.736951
sgKLB vs. sgFGFR1/3/4	-0.454	-0.7703 to -0.1377	Yes	**	0.00102
sgFGFR1 vs. sgFGFR3	0.29	-0.02631 to 0.6063	No	ns	0.093983
sgFGFR1 vs. sgFGFR4	2.494	2.178 to 2.81	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR1/3	0.602	0.2857 to 0.9183	Yes	****	0.00001
sgFGFR1 vs. sgFGFR1/4	2.222	1.906 to 2.538	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR3/4	3.67	3.354 to 3.986	Yes	****	<0.000001
sgFGFR1 vs. sgFGFR1/3/4	3.38	3.064 to 3.696	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR4	2.204	1.888 to 2.52	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR1/3	0.312	-0.004307 to 0.6283	No	ns	0.055633
sgFGFR3 vs. sgFGFR1/4	1.932	1.616 to 2.248	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR3/4	3.38	3.064 to 3.696	Yes	****	<0.000001
sgFGFR3 vs. sgFGFR1/3/4	3.09	2.774 to 3.406	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/3	-1.892	-2.208 to -1.576	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/4	-0.272	-0.5883 to 0.04431	No	ns	0.140215
sgFGFR4 vs. sgFGFR3/4	1.176	0.8597 to 1.492	Yes	****	<0.000001
sgFGFR4 vs. sgFGFR1/3/4	0.886	0.5697 to 1.202	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR1/4	1.62	1.304 to 1.936	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR3/4	3.068	2.752 to 3.384	Yes	****	<0.000001
sgFGFR1/3 vs. sgFGFR1/3/4	2.778	2.462 to 3.094	Yes	****	<0.000001
sgFGFR1/4 vs. sgFGFR3/4	1.448	1.132 to 1.764	Yes	****	<0.000001
sgFGFR1/4 vs. sgFGFR1/3/4	1.158	0.8417 to 1.474	Yes	****	<0.000001
sgFGFR3/4 vs. sgFGFR1/3/4	-0.29	-0.6063 to 0.02631	No	ns	0.093983

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