Support Information for:

Matrix-Augmented Pooling Strategy for High Throughput Target Deconvolution Reveals Cell-Specific Drug-Target Interactions

Supplementary figure & captions

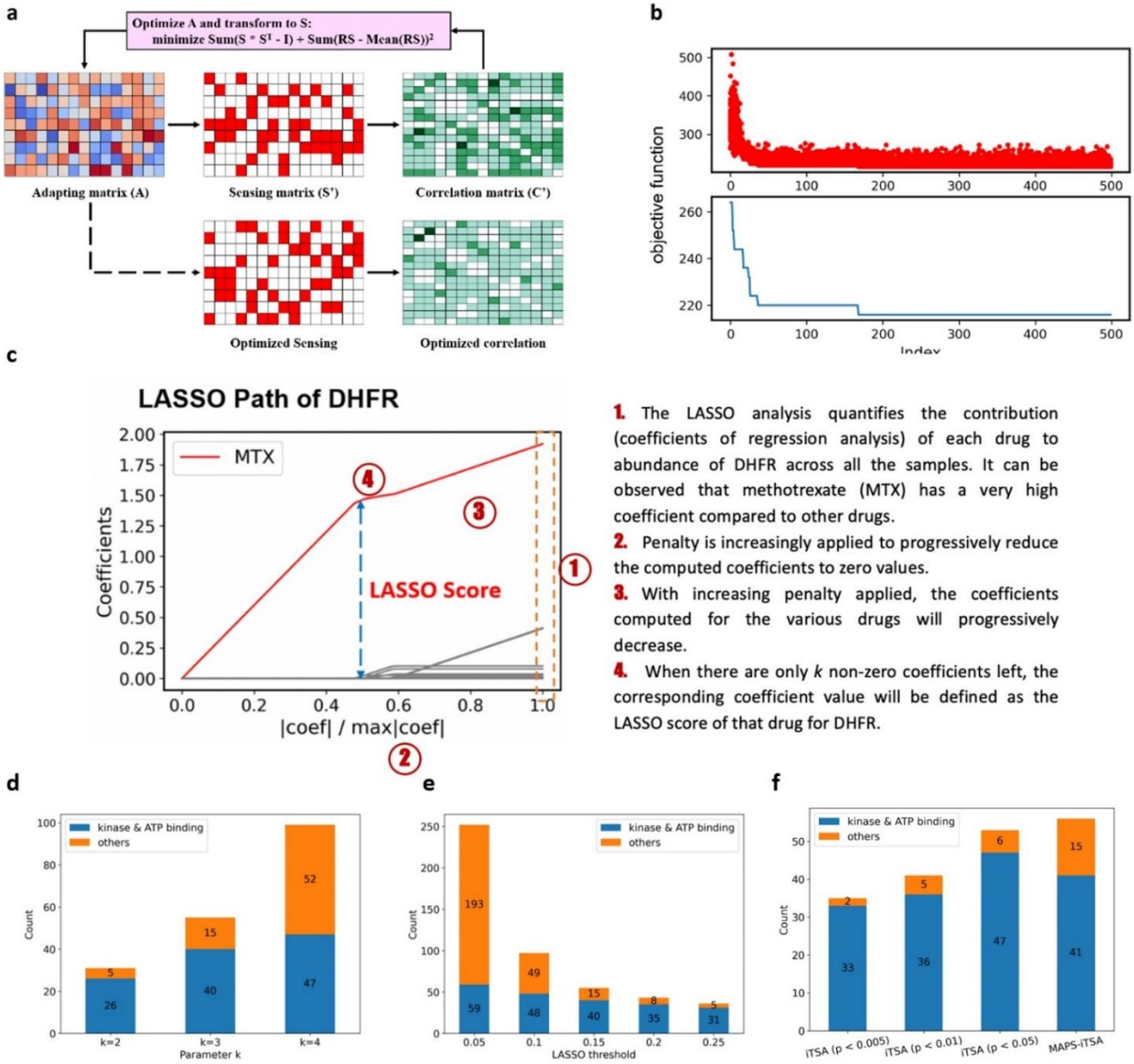

Fig. S1. Conception description, parameter optimization, and performance comparison of MAPS-iTSA algorithm, Related to STAR Methods. (a) The conceptual diagram of sensing matrix generation; (b) The result curve of optimization of genetic algorithm of sensing matrix generation; (c) The schematic diagram of how the LASSO score is computed. (d-e) The optimization results of the parameters of LASSO analysis based on kinase identification with HepG2 cell line. i.e., number of identified kinases and false positives with different parameter *k* (d), and number of identified kinases and false positives with different threshold of LASSO score (e); (f) Kinase identification performance comparison between MAPS-iTSA and iTSA with different p-value cutoff (Blue: Number of identified kinase and ATP-binding proteins, treated as true positive; Orange: Number of other identified proteins, treated as false positive).

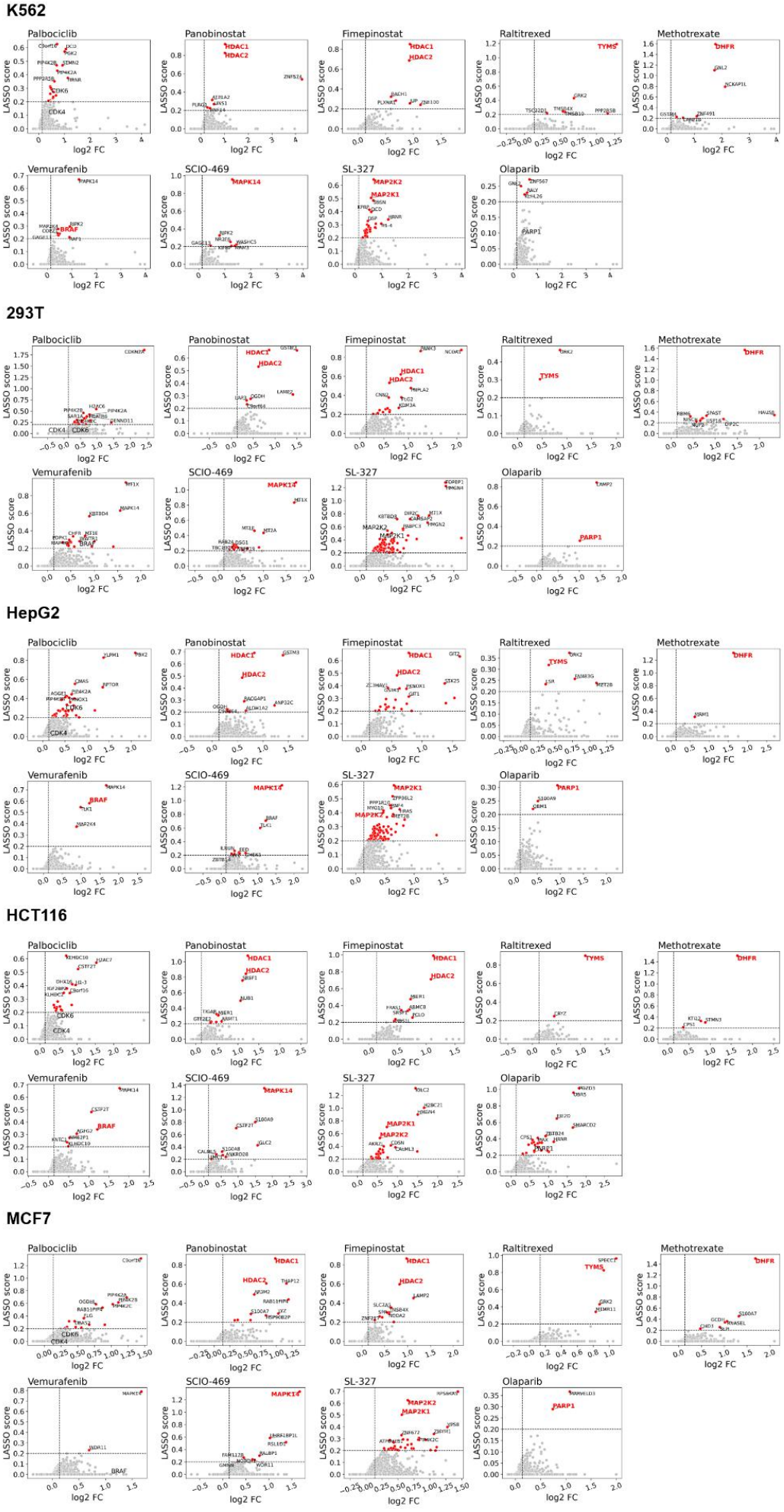

Fig. S2. Scatter plot of drug target deconvolution across cell-lines results of validation set, Related to Figure 2.

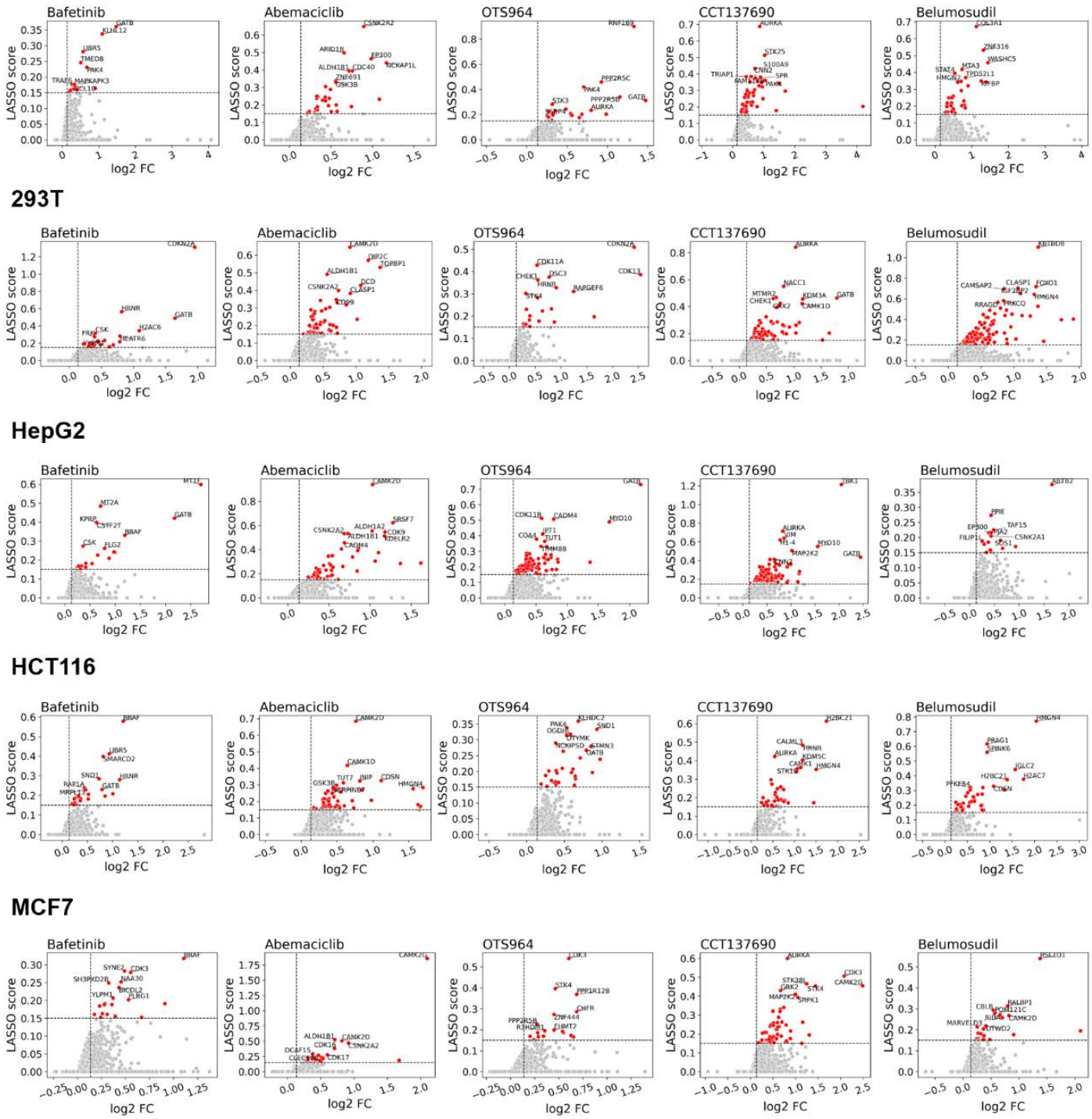

Fig. S3. Scatter plot of drug target deconvolution across cell-lines results of exploratory set, Related to Figure 4.

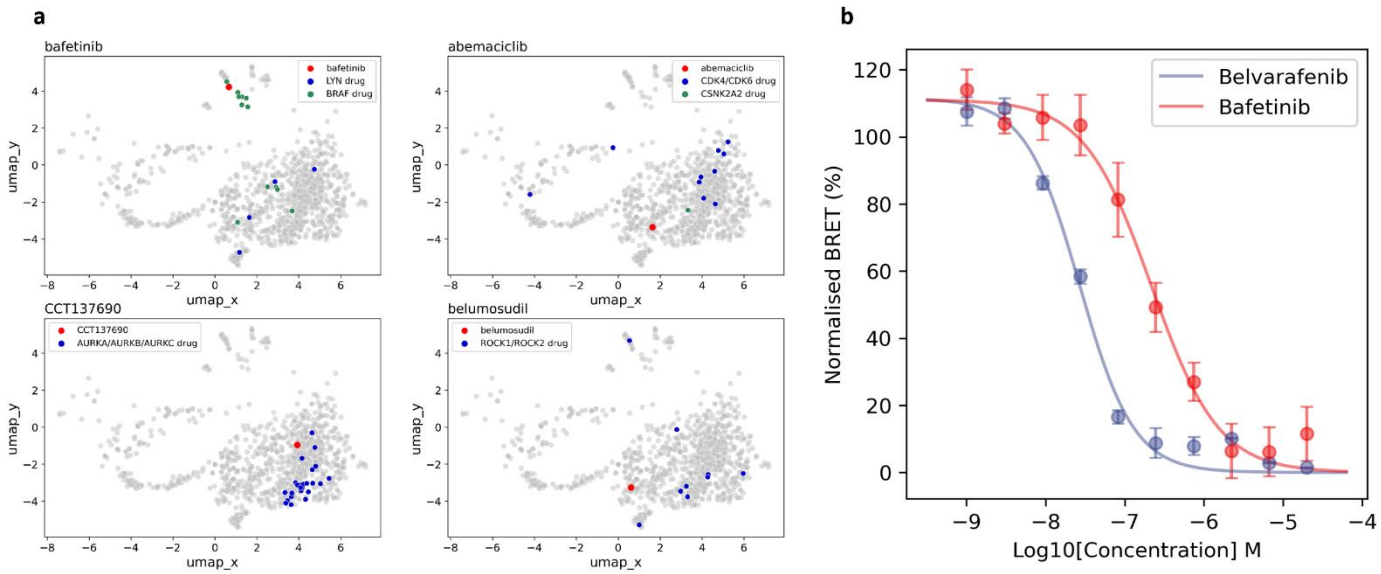

Fig. S4. Basis of off-target drug speculation and verification, related to STAR Methods. (a) U-map visualization of the drug response patterns of 578 cancerous cell lines, which indicates the selected exploratory drugs have the different killing profile from the other drugs with the same designed targets; (b) Dose-response curve for NanoBERT in-vitro off-target validation of bafetinib-BRAF V600E.

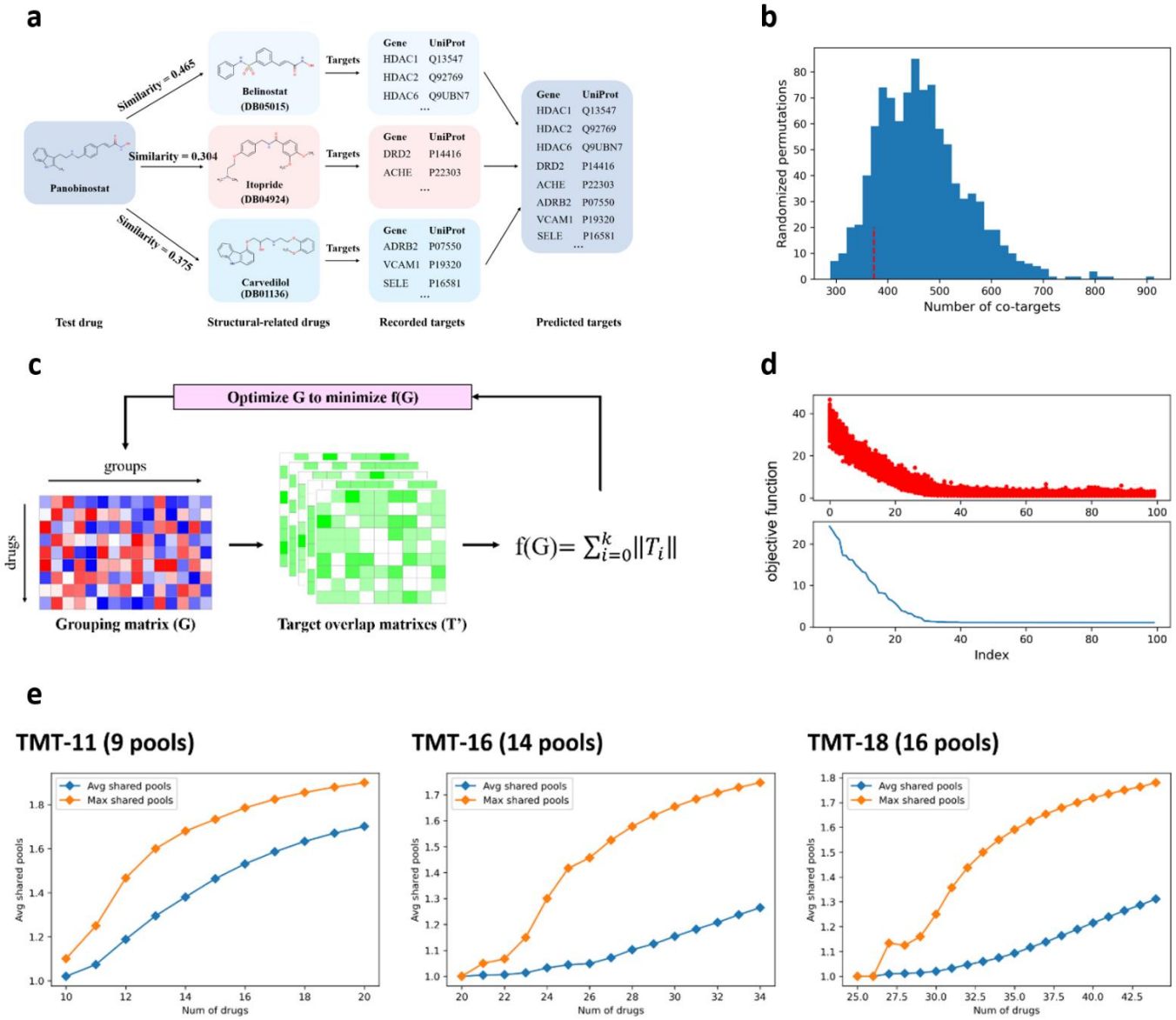

Fig. S5. Concept and methods for promoting application prospects of MAPS-iTSA, related to STAR Methods. (a) The schematic diagram of drug target prediction based on structural similarity used for drug grouping. (b) Comparison between the results of drug grouping algorithm and random grouping. The histogram represents the frequency distribution of the number of co-targets of random grouping. The red line indicates the number of co-targets achieved through the drug grouping method. Based on the results, it is evident that the optimized result outperforms the majority of random grouping situations. This suggests that the drug grouping algorithm is effective to reduce the co-targets within drug groups. (c) The conceptual diagram of drug grouping algorithm; (d) The result curve of optimization of genetic algorithm of drug grouping. (e) Scatter plots of different TMT channels between the number of test drugs and the shared pools per drug.

Supplementary Tables

Table S1. List of drugs used in the experiments, related to STAR Methods.

PubChem	Drug	Known target	Reference	Category
5330286	Palbociclib	CDK4, CDK6	EMBO J. 37, 1–19 (2018)	verification
6918837	Panobinostat	HDAC1, HDAC2	Nat. Chem. Biol. 12, 908–910 (2016)	verification
135400182	Raltitrexed	TYMS	Science 341, 84–87 (2013)	verification
126941	Methotrexate	DHFR	Science 341, 84–87 (2013)	verification
42611257	Vemurafenib	BRAF	Science 346, 1255784 (2014)	verification
54575456	Fimepinostat	HDAC1	J. Med. Chem. 63, 4256–4292 (2020)	verification
23725625	Olaparib	PARP1	Science 341, 84–87 (2013)	verification
11387605	Bafetinib	LYN	Nat. Cancer 1, 235–248 (2020)	exploratory
9871074	SCIO-469	MAPK14	Sci. Transl. Med. 11, eaaw8412 (2019)	exploratory
89675898	OTS964	TOPK	Sci. Transl. Med. 11, eaaw8412 (2019)	exploratory
9549284	SL-327	MEK1, MEK2	Nat. Cancer 1, 235–248 (2020)	exploratory
46220502	Abemaciclib	CDK4, CDK6	Nat. Cancer 1, 235–248 (2020)	exploratory
25154041	CCT137690	AURKA, AURKB, AURKC	Nat. Cancer 1, 235–248 (2020)	exploratory
11950170	Belumosudil	ROCK1, ROCK2	Nat. Cancer 1, 235–248 (2020)	exploratory
7251185	Parthenolide	NFKB1	Nat. Cancer 1, 235–248 (2020)	exploratory
3385	Staurosporine	TYMS	Science 341, 84–87 (2013)	verification

Table S2. Sensing matrix of the 9*15 experiment, related to STAR Methods.

Pool ID	Palbociclib	Panobinostat	Raltitrexed	Methotrexate	Vemurafenib	Fimepinostat	Olaparib	Bafetinib	SCIO-469	OTS964	SL-327	Abemaciclib	CCT137690	Belumosudil	Staurosporine / Parthenolide
1		√	√		√				√					√	
2					√	√		√	√		√				
3							√		√				√	√	√
4	√			√		√						√		√	
5	√	√					√	√		√					
6				√	√			√					√		√
7	√		√							√	√		√		
8			√			√				√		√			√
9		√		√			√				√	√			

Table S3. Raw data of Nanobert in vitro validation, related to Figure 5.

NanoBREAT BRAF										
		Belvarafenib（mBU）			Bafetinib（mBU）			Abemaciclib（mBU）		
Conc.	Conc. (log 10)	Response 1	Response 2	Response 3	Response 1	Response 2	Response 3	Response 1	Response 2	Response 3
0	#NUM!	3.91367133	4.15745458	3.70074564	4.29271532	5.0408172	3.3595527	4.44636131	3.88059691	4.20568858
1.016E-09	-8.9931063	4.92645029	4.06562082	4.22605651	4.29018475	5.12624398	4.58617954	4.89076345	3.86630863	5.35594291
3.04E-09	-8.5171264	6.41226044	4.1876454	3.80563734	4.37410354	4.70702356	4.26566459	4.99376502	3.71102745	4.5109384
9.14E-09	-8.0390538	3.67631771	3.95867175	4.0162304	4.10877103	5.28617288	4.99312735	4.49045192	4.06233281	4.62495775
2.74E-08	-7.5622494	5.64755568	3.42218132	3.64201137	3.90053621	5.48002447	4.32560794	4.20568345	4.17429005	4.89290655
8.24E-08	-7.0840728	2.6234896	2.47884406	2.76955391	3.60372325	4.93595232	5.16750744	4.23085903	5.04005282	4.48608204
2.46E-07	-6.6090649	1.97017143	1.400082	1.95155146	3.41564127	4.3101304	5.08324032	4.7155405	4.36087163	3.98765522
0.00000074	-6.1307683	0.7726647	0.3566775	0.51964133	2.90107792	3.92976414	3.30299941	4.95897982	3.78074991	6.63864627
0.00000222	-5.653647	1.77357143	0.55711707	0.98475173	2.24164584	2.73765951	2.72037654	4.67373743	4.18873093	5.99442342
0.00000666	-5.1765258	0.27011649	0.0647367	0.643571	0.23528935	0.94095959	0.56072518	5.06167616	4.77996852	5.77392157
0.00002	-4.69897	1.12021459	0.48017526	0.09472179	0.17752265	1.27839232	0.76452055	4.76435921	5.82758129	5.46516649

(Continue) Table S3. Raw data of Nanobert in vitro validation, related to Figure 5.

NanoBREAT BRAF_V600E										
		Bafetinib（mBU）			Abemaciclib（mBU）			Belvarafenib（mBU）		
Conc.	Conc. (log 10)	Response 1	Response 2	Response 3	Response 1	Response 2	Response 3	Response 1	Response 2	Response 3
0	#NUM!	5.33638492	5.53715947	5.74809842	6.52772549	4.80861318	4.61630598	6.18910475	5.52460832	5.2892683
1.016E-09	-8.9931063	6.07896771	5.91532471	6.97722597	6.08293452	5.39010574	5.27806244	5.40874768	6.17269327	5.46227386
3.04E-09	-8.5171264	5.39190522	5.99447881	5.90242629	6.19982835	5.86573776	4.97257656	5.77847081	6.16186375	5.57299865
9.14E-09	-8.0390538	5.2048433	6.30915437	6.08070695	6.52705614	5.22476852	6.11906738	2.4511827	4.8740865	4.45457095
2.74E-08	-7.5622494	5.59297462	6.29859681	5.28191939	5.88861626	5.39667028	5.64593773	2.03523839	3.10841126	3.2001538
8.24E-08	-7.0840728	3.86437925	5.35295135	4.29116974	6.23030069	5.57039729	5.71447366	0.03125875	1.02366879	0.7676262
2.46E-07	-6.6090649	2.18990768	3.25301082	2.74688389	5.4425841	5.02745995	5.78499709	-0.1859074	0.73042948	0.22908728
0.00000074	-6.1307683	1.0178843	1.77013328	1.71893831	6.11209876	5.6023338	5.78441447	-0.3336585	0.58008964	0.27599117
0.00000222	-5.653647	-0.0225809	0.9859748	0.10193503	6.50798001	4.7763717	5.71491838	-0.6425143	0.57539389	0.52284339
0.00000666	-5.1765258	-0.0614731	0.89476916	0.19973177	7.21915738	6.14606683	7.30966575	-0.3404072	0.15181606	0.15955778
0.00002	-4.69897	0.31041542	1.27076932	0.32975827	7.16622475	7.40829925	6.98164257	-0.029289	0.08480661	0.07154295

Table S4. Raw data of kinase hotspot target validation assay, related to Figure 5.

Kinase Profiling Report for:		**BRAF**					**[ATP](μM):**	**10**
Conc.(M)	**Abemaciclib**	**Abemaciclib**	**Abemaciclib**	**Bafetinib**	**Bafetinib**	**Bafetinib**	**GW5074**	**GW5074 Conc.(M)**
1.00E-04	80715	84419	79180	-2031	-4443	-5375	-6052	**2.00E-05**
3.33E-05	149786	147694	156034	-1682	-3885	-5622	-4800	**6.67E-06**
1.11E-05	226330	255462	267275	-4434	-6823	-5568	-4421	**2.22E-06**
3.70E-06	358606	346775	385519	-6456	-6266	-3820	4038	**7.41E-07**
1.23E-06	409213	413339	430776	-848	-1857	-2842	16162	**2.47E-07**
4.12E-07	456265	441384	489258	24033	23657	23139	42661	**8.23E-08**
1.37E-07	525557	481983	539703	102191	112396	109556	143045	**2.74E-08**
4.57E-08	552216	544026	572315	276285	259062	256632	365438	**9.14E-09**
1.52E-08	516129	545563	562423	391487	383164	368225	510805	**3.05E-09**
5.08E-09	539302	534184	562684	509419	494436	477746	595780	**1.02E-09**
DMSO	595696	584900	611632	598580	606738	601776	571292	**DMSO**

(Continue) Table S4. Raw data of kinase hotspot target validation assay, related to Figure 5.

Kinase Profiling Report for:		**CK2a2**					**[ATP](μM):**	**10**
Conc.(M)	**Abemaciclib**	**Abemaciclib**	**Abemaciclib**	**Bafetinib**	**Bafetinib**	**Bafetinib**	**Staurosporine**	**Stauro Conc.(M)**
1.00E-04	7297	8941	5444	458598	438803	497560	78271	**2.00E-05**
3.33E-05	6042	2659	5092	683213	645269	691405	147138	**5.00E-06**
1.11E-05	10836	12563	9963	915233	969850	949722	367327	**1.25E-06**
3.70E-06	25017	21513	25367	923466	606108	912391	579509	**3.13E-07**
1.23E-06	57688	57174	61108	1093698	1014825	1059729	879043	**7.81E-08**
4.12E-07	135849	121083	133100	1086069	1086189	1050479	979504	**1.95E-08**
1.37E-07	322631	305443	284817	1144749	1143367	1123120	1074994	**4.88E-09**
4.57E-08	539792	491428	494882	1069922	1089055	1118575	1029417	**1.22E-09**
1.52E-08	780197	723043	744052	1050756	1042893	1075383	1037887	**3.05E-10**
5.08E-09	971791	923850	921151	1069341	1026662	1060508	1028017	**7.63E-11**
DMSO	1088765	1169281	1113277	1121022	1086907	1113102	1095674	**DMSO**