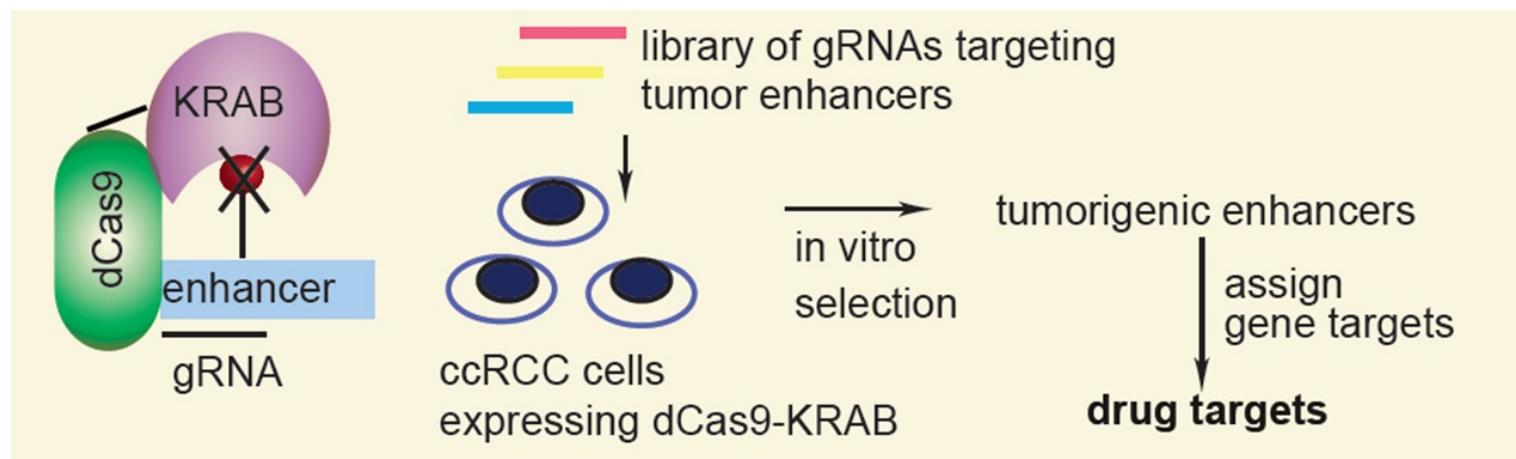


## Project 1: Identification of oncogenic genes through functional screening of gained enhancers in ccRCC

Gained enhancer screening using dCas9-KRAB gene repression system



A total of 12,330 gRNAs targeting the top 200 gained enhancers for screening (on average 62 gRNAs per enhancer)

Initial screening done in Duke and National University of Singapore

## Screening results

A498 cells

1	enhancerID	A498 ctrl_1 R2	A498 ctrl_2 R2	A498 ctrl_3 R2	krab_gain_a498	krab_gain_a498	krab_gain_a498
2	chr1_224352252_2	54	36	64	6	10	28
3	chr1_232901432_2	160	90	105	68	50	64
4	chr10_102107689_	77	149	100	32	22	44
5	chr10_124061302_	179	95	130	76	42	76
6	chr10_124061422_	40	26	32	0	8	14
7	chr10_124061494_	114	108	94	84	43	26
8	chr10_126077434_	100	58	106	30	40	18
9	chr10_48573651_4	37	63	26	6	0	3
10	chr10_6217527_62	38	80	56	27	11	18
11	chr11_126018256_	132	97	66	48	22	30
12	chr11_74384148_7	77	137	88	48	37	54
13	chr12_125259836_	180	150	74	81	26	54
14	chr12_89560907_8	157	107	140	65	77	92
15	chr13_51161849_5	326	268	312	179	197	212
16	chr13_51161888_5	148	54	120	58	36	36
17	chr14_92942128_9	71	71	50	38	18	24
18	chr14_93006803_9	130	152	102	72	32	68
19	chr15_102152141_	35	94	58	17	30	14
20	chr15_102154630_	71	57	38	16	10	0
21	chr15_65162719_6	120	164	128	84	66	82
22	chr15_66871265_6	68	82	34	22	25	19
23	chr15_68190757_6	181	228	162	66	116	134
24	chr16_47081480_4	282	238	246	114	118	225
25	chr16_69051416_6	113	118	34	5	2	55
26	chr16_86774476_8	36	34	50	18	12	12
27	chr17_1101572_11	84	141	106	81	28	50
28	chr17_36209033_3	194	338	137	130	91	130
29	chr19_4691062_46	56	64	54	24	22	18
30	chr19_4816187_48	140	69	69	52	35	50
31	chr2_123066615_1	127	96	78	38	36	52
32	chr2_123461948_1	204	146	198	124	94	114
33	chr2_218582436_2	250	152	157	70	112	121
34	chr2_219827958_2	194	150	148	78	94	125
35	chr2_28391523_28	76	20	12	0	4	10
36	chr2_65065753_65	118	175	216	114	62	117
37	chr2_85153852_85	48	20	90	0	10	24
38	chr20_34380927_3	36	54	60	34	4	0

786O cells

1	enhancerID	gain_ctrl_1	gain_ctrl_2	gain_ctrl_3	krab_gain_7l	krab_gain_7l	krab_gain_7l
2	chr1_224357203	22	72	74	0	2	0
3	chr1_231939402	42	68	20	0	0	4
4	chr1_59509230	56	68	6	0	0	0
5	chr10_14509073	66	16	74	0	2	0
6	chr10_3514530	89	14	36	3	0	0
7	chr10_73625276	118	27	154	11	0	8
8	chr10_99630060	41	17	19	0	0	0
9	chr11_69235207	77	43	23	0	0	0
10	chr11_74384256	52	122	185	10	0	0
11	chr12_12138252	81	131	49	0	4	5
12	chr12_25538833	53	158	120	7	0	1
13	chr12_52600297	123	160	204	10	2	9
14	chr12_89554229	51	82	52	0	3	8
15	chr14_69330135	20	20	26	0	0	0
16	chr14_92940423	148	147	153	17	0	10
17	chr16_47081339	48	51	63	8	0	3
18	chr17_29277367	120	47	12	0	0	0
19	chr17_29280245	114	98	12	0	4	8
20	chr18_56030425	84	64	98	4	0	0
21	chr19_4691079	73	82	48	0	0	7
22	chr19_54352653	91	74	110	0	3	0
23	chr2_18595289	98	91	58	0	6	0
24	chr2_218558611	50	30	24	0	0	0
25	chr2_219828203	247	153	274	15	13	14
26	chr2_235096939	120	78	114	6	0	9
27	chr2_241519352	66	58	34	0	1	3
28	chr2_28391592	172	74	207	0	6	4
29	chr2_58339545	20	18	33	0	0	0
30	chr20_1518201	89	74	106	0	0	0
31	chr3_126701126	85	63	17	7	0	2
32	chr3_138582521	142	118	136	0	10	6
33	chr3_194002922	130	165	120	1	8	5
34	chr3_194003736	28	32	2	0	0	0
35	chr4_25912016	30	63	24	0	0	0
36	chr5_111146070	129	104	51	9	0	5
37	chr5_1474857_1	16	8	13	0	0	0
38	chr5_1474859_1	35	14	26	0	1	0

786O cells: 54 dropouts  
(tumorigenic enhancers)



A498 cells: 78 dropouts  
(tumorigenic enhancers)

## Assigning the dropout enhancer targeted genes

**78** tumorigenic enhancers  
(dropouts) identified in A498 cells

**54** tumorigenic enhancers  
(dropouts) identified in 786O cells

↓  
Assigning enhancer  
targeted genes

123 genes in A498 cells

93 genes in 786O cells

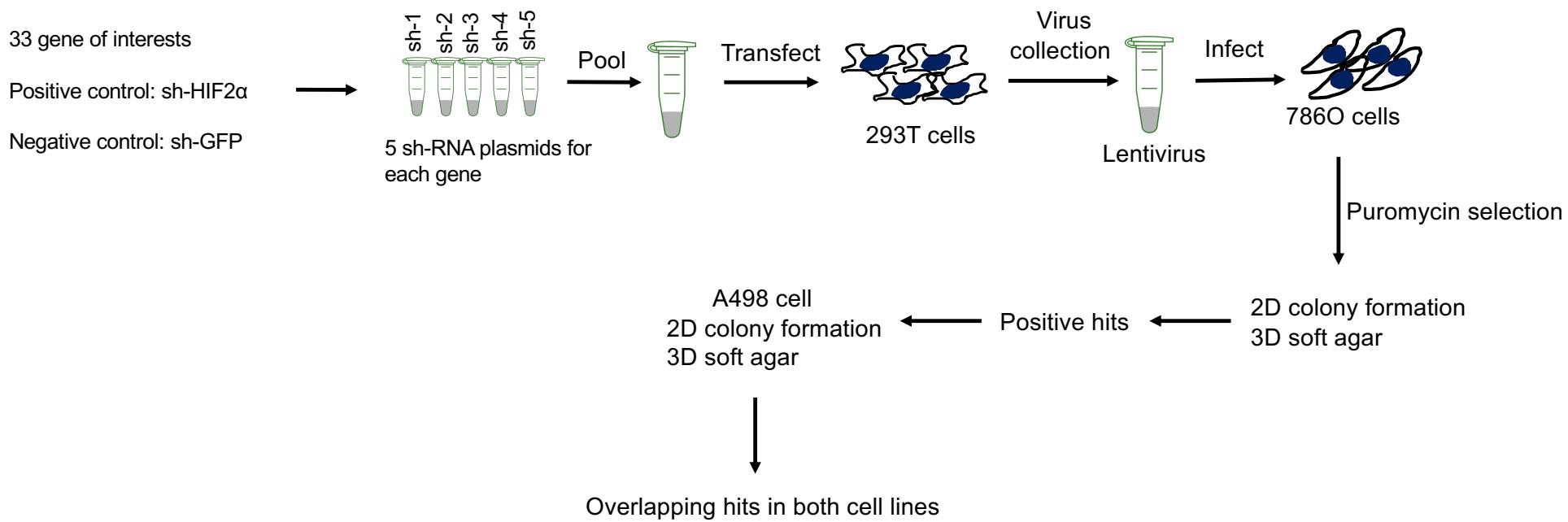
33 overlapping genes in both cell lines  
(potential tumor promoting genes in ccRCC)

TP53BP2	CPN2	SLC24A4	COL23A1
CHRDL2	STARD4	NETO2	SMPDL3A
KITLG	SLC6A3	DPP9	PPEF1
RIN3	LPCAT1	TNS1	
DNAJA2	PHYKPL	FEV	
C19orf10	PHKA2	FOSL2	
TNP1	DEGS1	CHCHD6	
CDK5R2	SIPA1L2	HES1	
RBKS	POLD3	NREP	
PLXNA1	DUSP6	MRPL36	

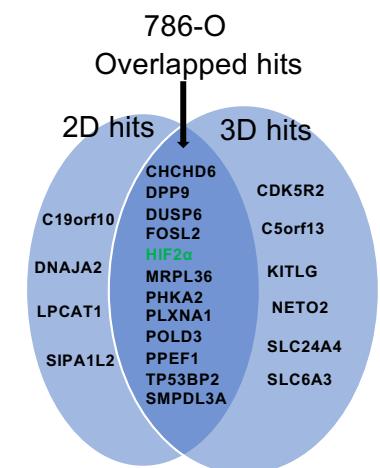
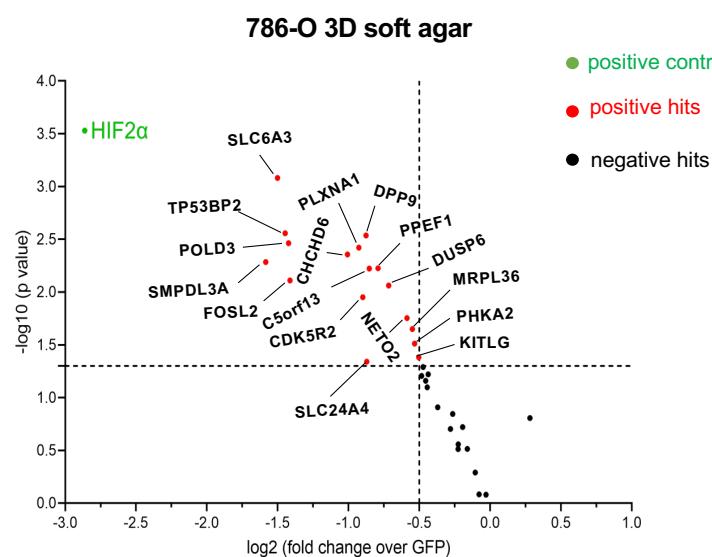
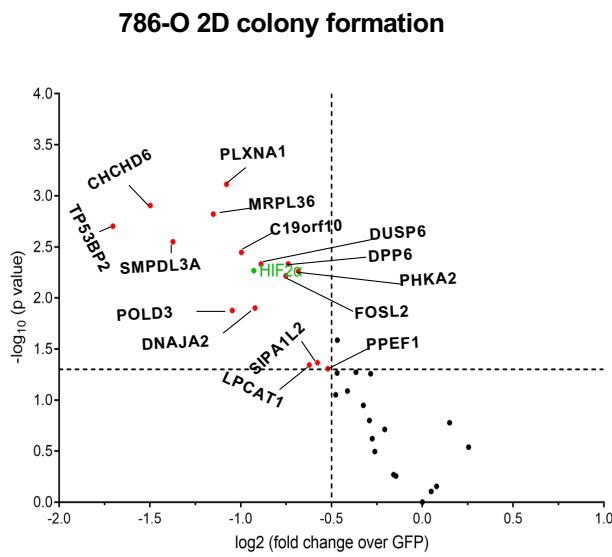
→ Gene list for  
functional validation

## Functional validation of the 33 putative tumorigenic genes

Sh-RNA mediated gene knockdown screening:

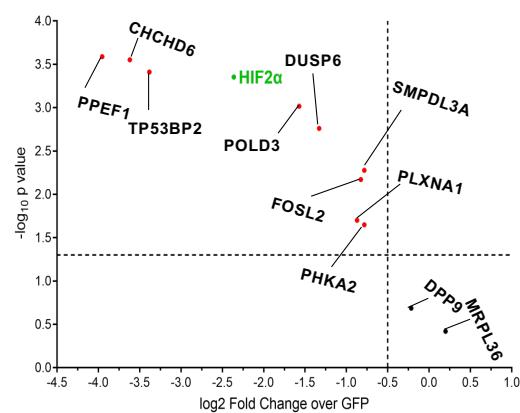


## Pool sh-RNA screening results in 786-O cells

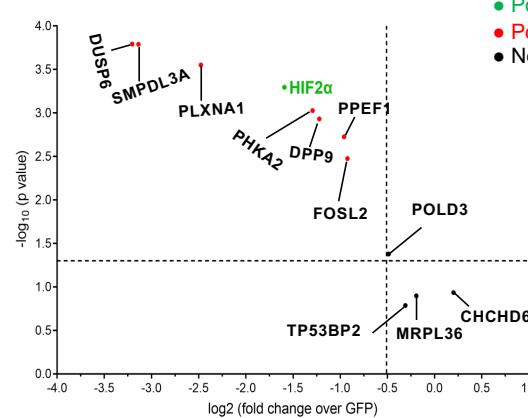


Screen the 786-O positive hits (12 genes) in A498 cells

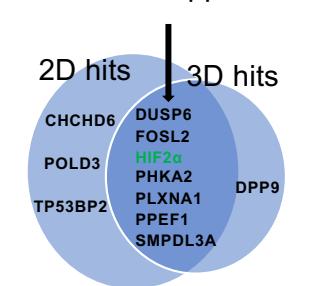
A498 2D colony formation



A498 3D soft agar



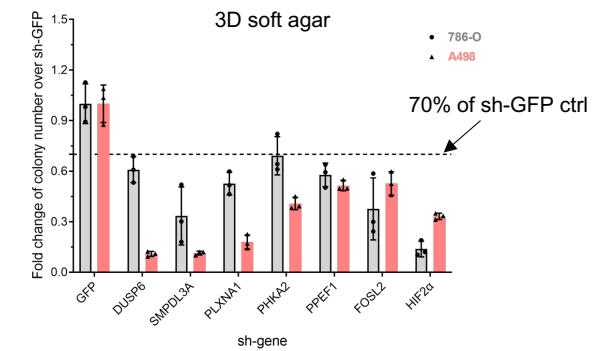
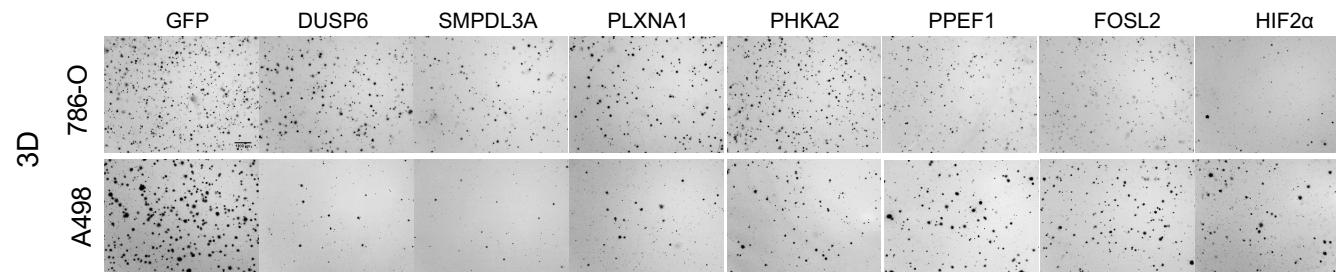
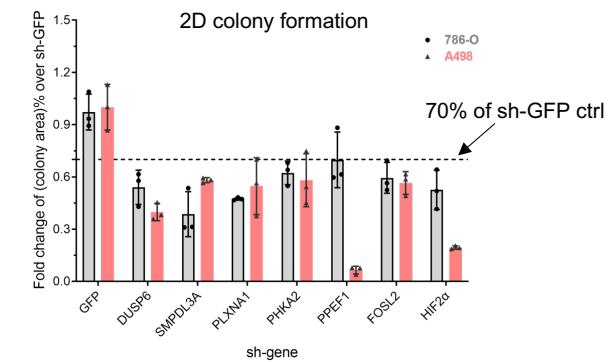
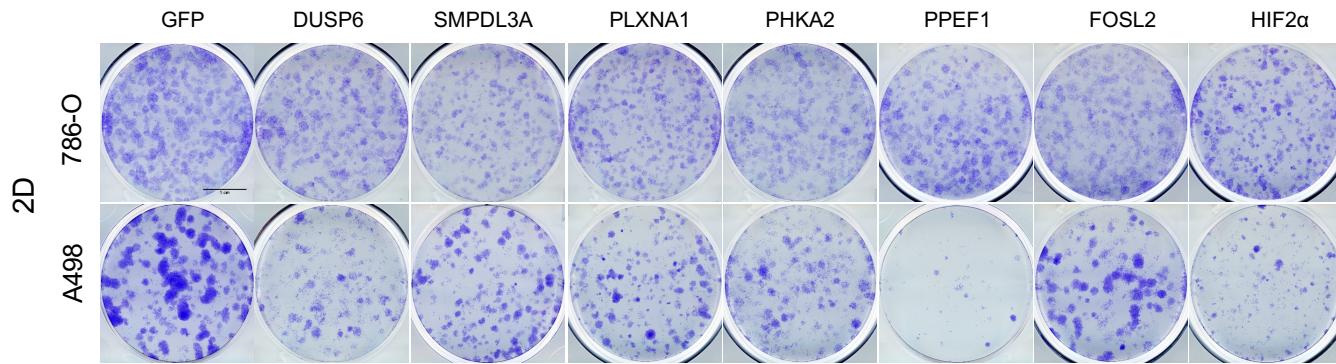
A498 overlapped hits



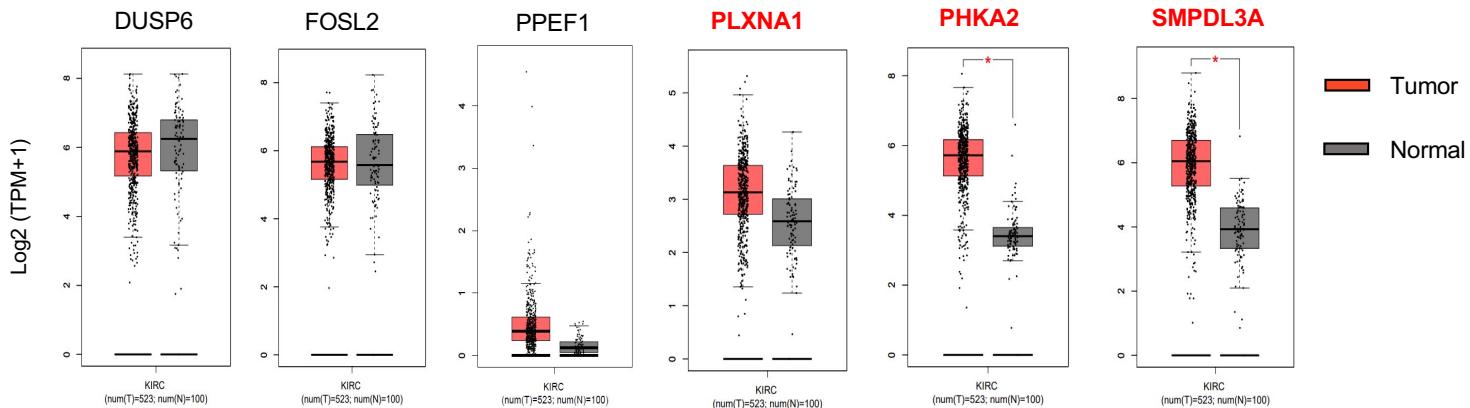
**Positive hits in both 786-O and A498 cells:**

DUSP6, SMPDL3A, PLXNA1, PHKA2, PPEF1, FOSL2

## Representative results of the 6 positive hits



## Expression of the 6 positive hits in ccRCC tumors (TGCA data from GEPIA)



**Choosing PLXNA1, PHKA2 and SMPDL3A as my candidate genes!!**

**PLXNA1**, also called Plexin A1, Co-receptor for semaphorins.

➤ Cancer Biother Radiopharm. 2021 Dec 1. doi: 10.1089/cbr.2021.0336. Online ahead of print.

**Promising Therapeutic Targets in Kidney Renal Clear Cell Carcinoma: *PLXNA1* and *PLXNB3***

Can-Xuan Li <sup>1</sup>, Dan Long <sup>2</sup>, Quan Meng <sup>3</sup>

Affiliations + expand

PMID: 34851747 DOI: 10.1089/cbr.2021.0336

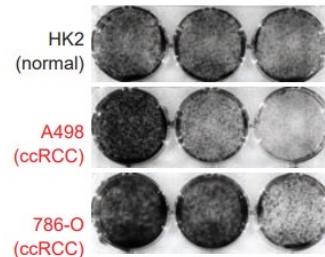
**SMPDL3A:** Sphingomyelin Phosphodiesterase Acid Like 3A or Acid sphingomyelinase-like phosphodiesterase 3a.

Has nucleotide phosphodiesterase activity with nucleoside triphosphates.

**VHL Deficiency Drives Enhancer Activation of Oncogenes in Clear Cell Renal Cell Carcinoma** 

Xiaosai Yao; Jing Tan; Kevin Junliang Lim; Joanna Koh; Wen Fong Ooi; Zhimei Li; Dachuan Huang; Manjie Xing; Yang Sun Chan; James Zhengzhong Qu; Su Ting Tay; Giovani Wijaya; Yue Ning Lam; Jing Han Hong; Ai Ping Lee-Lim; Peiyong Guan; Michelle Shu Wen Ng; Cassandra Zhengxuan He; Joyce Suling Lin; Tannistha Nandi; Aditi Qamra; Chang Xu; Swe Swe Myint; James O. J. Davies; Jian Yuan Goh; Gary Loh; Bryan C. Tan; Steven G. Rozen; Qiang Yu; Iain Bee Huat Tan; Christopher Wai Sam Cheng; Shang Li; Kenneth Tou En Chang; Puay Hoon Tan; David Lawrence Silver; Alexander Lezhava; Gertrud Steger; Jim R. Hughes; Bin Tean Teh ; Patrick Tan 

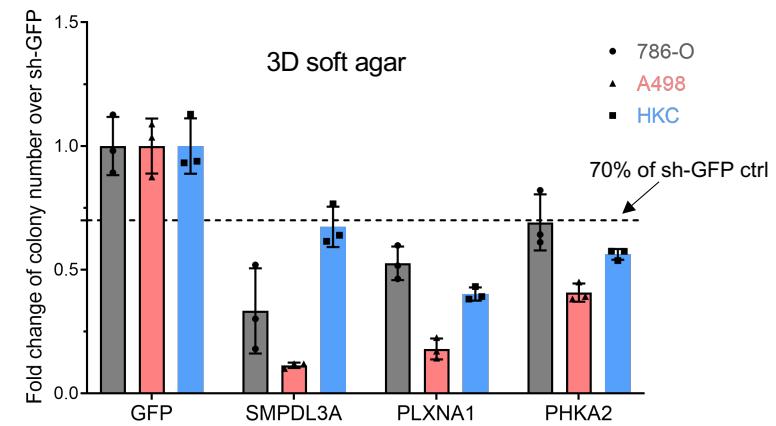
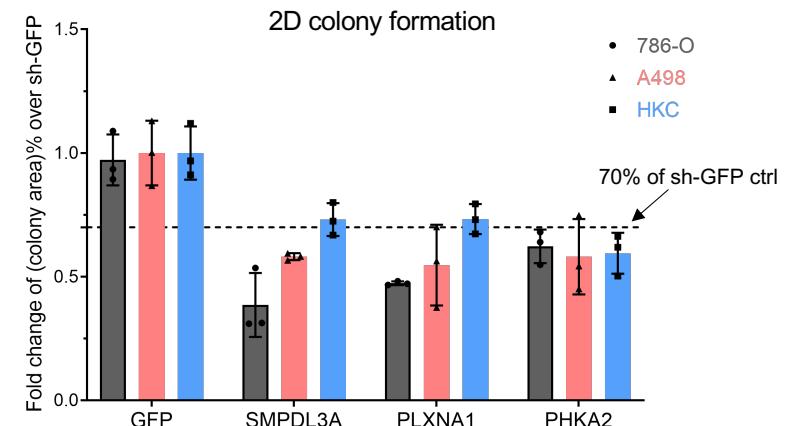
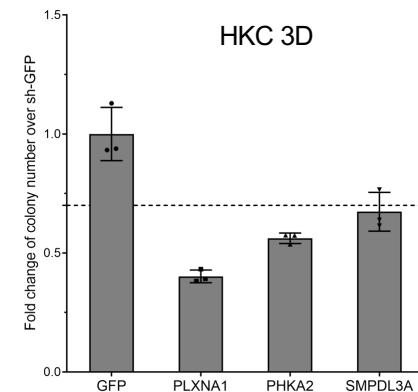
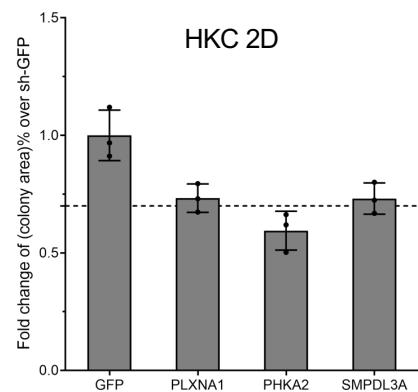
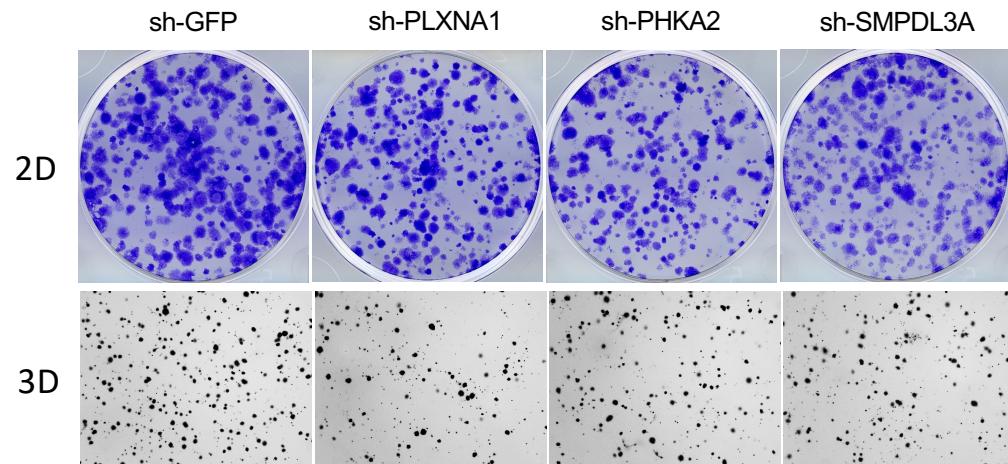
siRNA: NC SMPDL3A ZNF395



**PHKA2:** Phosphorylase Kinase Regulatory Subunit Alpha 2

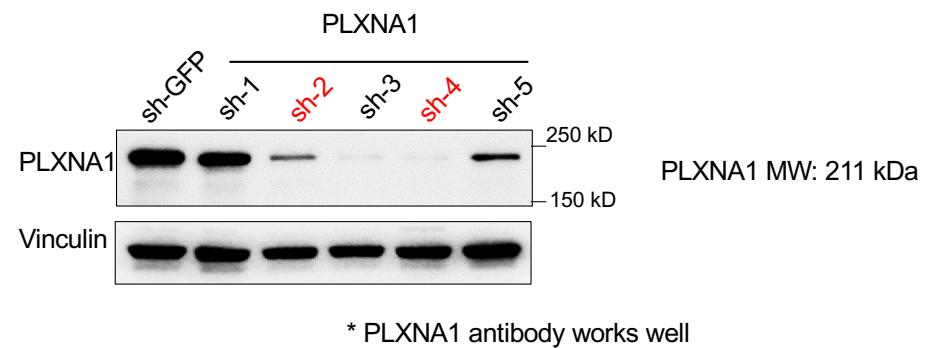
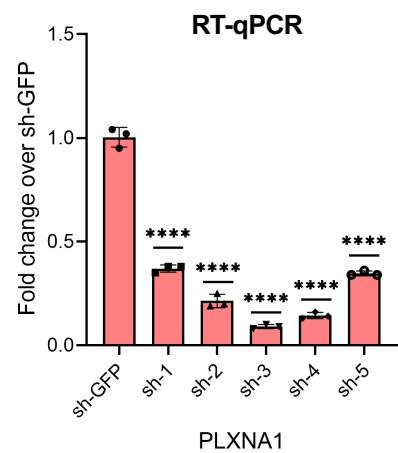
Involved in glycogen metabolism, no reports found in kidney cancer

## Pooled sh-RNA knockdown of PLXNA1, PHKA2 and SMPDL3A in HKC cells

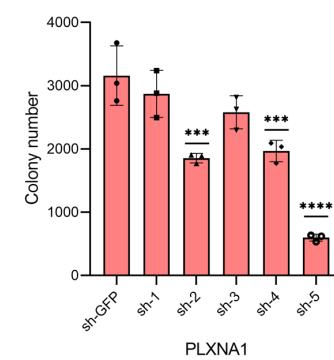
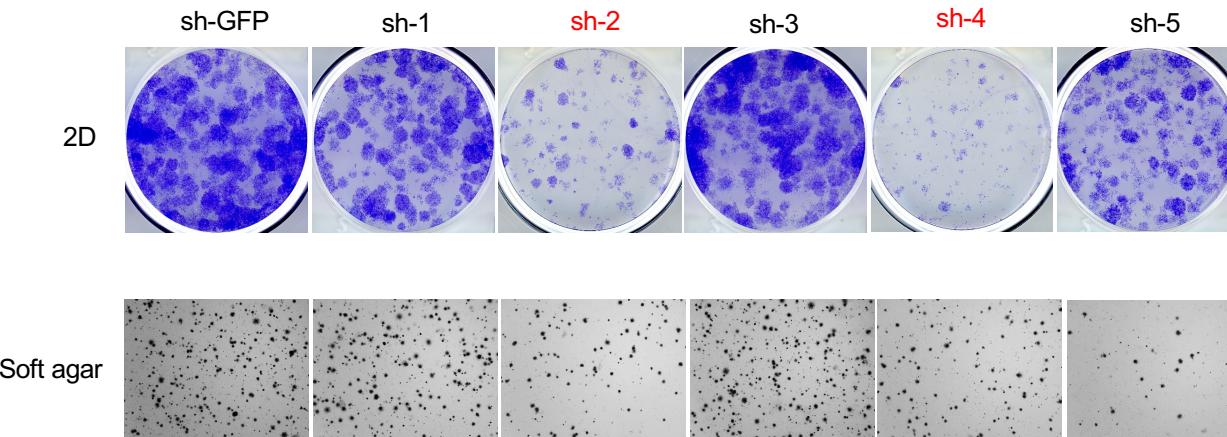


## Individual sh-RNA validation in 786-O cells

**PLXNA1**

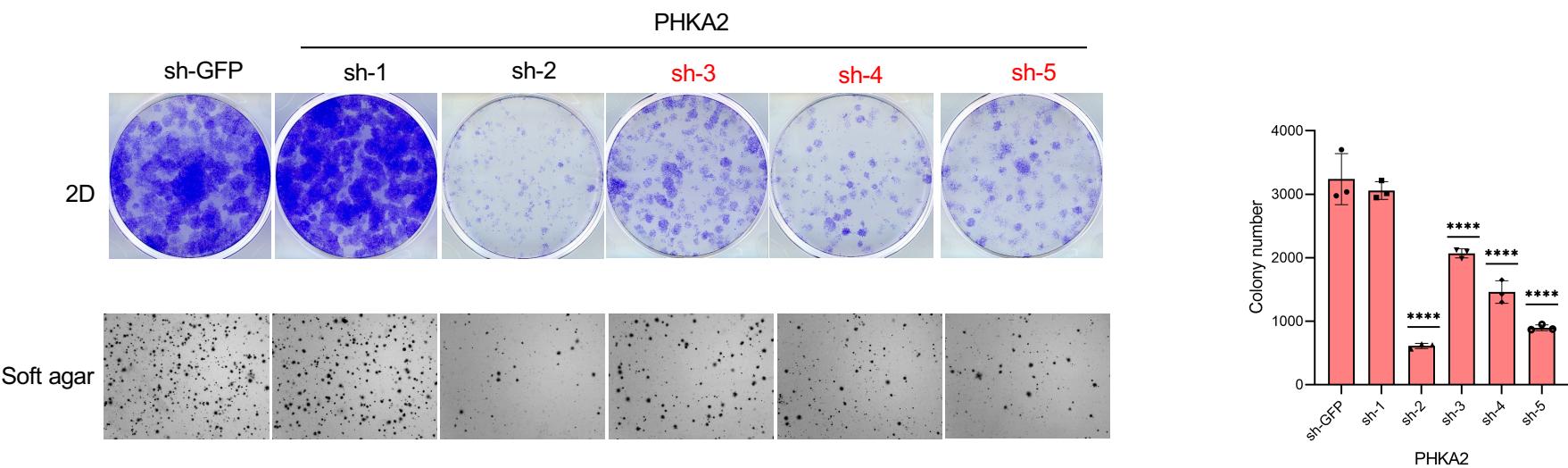
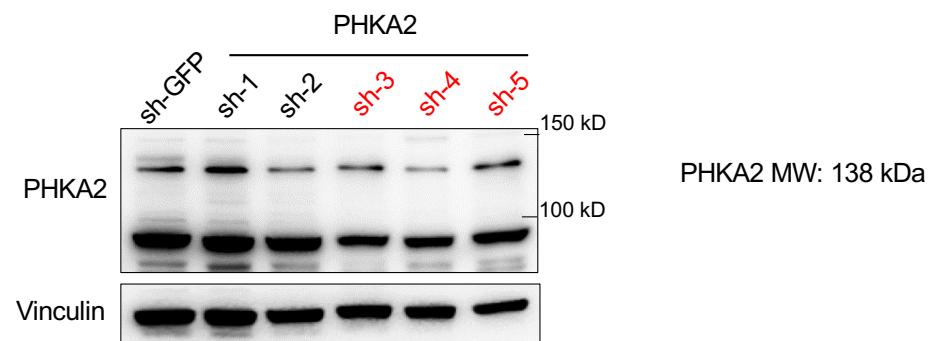
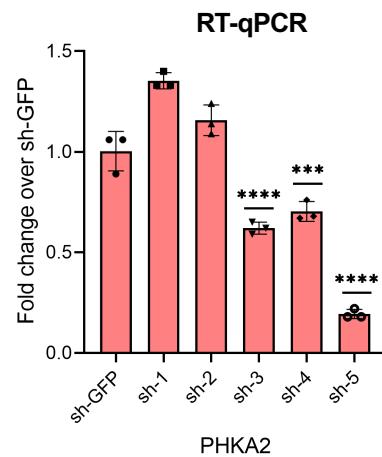


**PLXNA1**



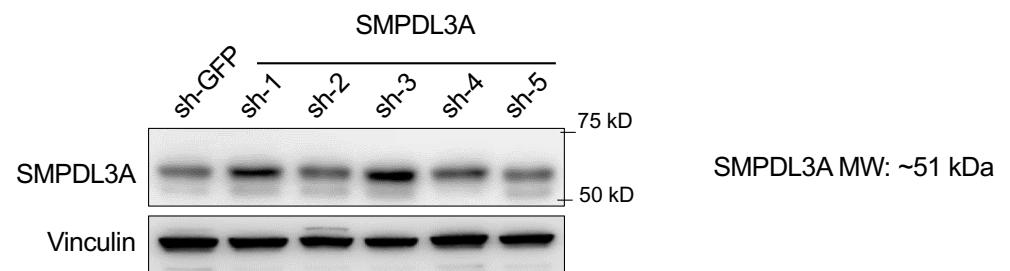
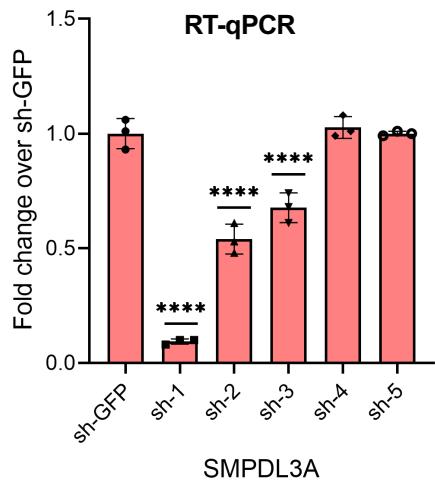
## Individual sh-RNA validation in 786-O cells

**PHKA2**

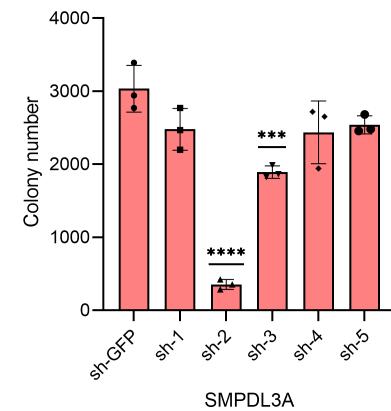
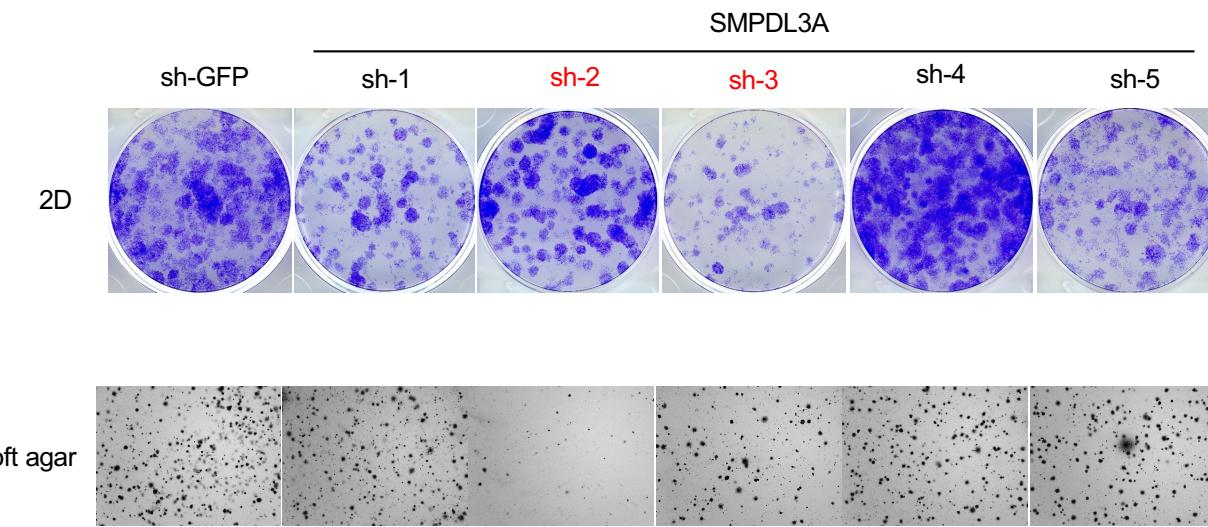


## Individual sh-RNA validation in 786-O cells

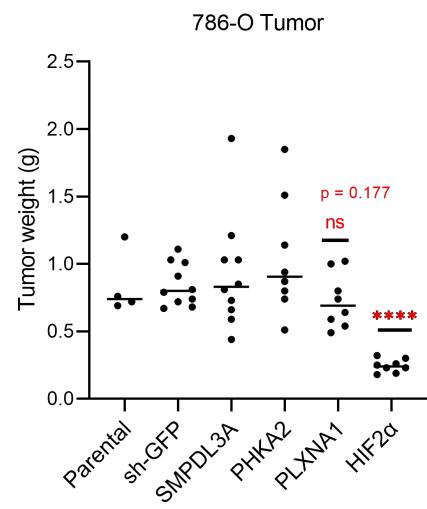
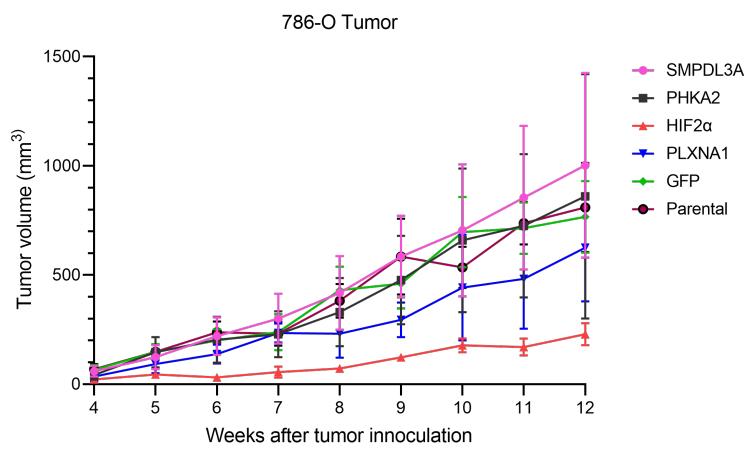
### SMPDL3A



\* Antibody does not work, needs to try with new antibody



#### *In vivo* 786-O tumor growth with pool shRNA knockdown of the 3 hit genes



Will try this again with individual sh-RNA or inducible sgRNA

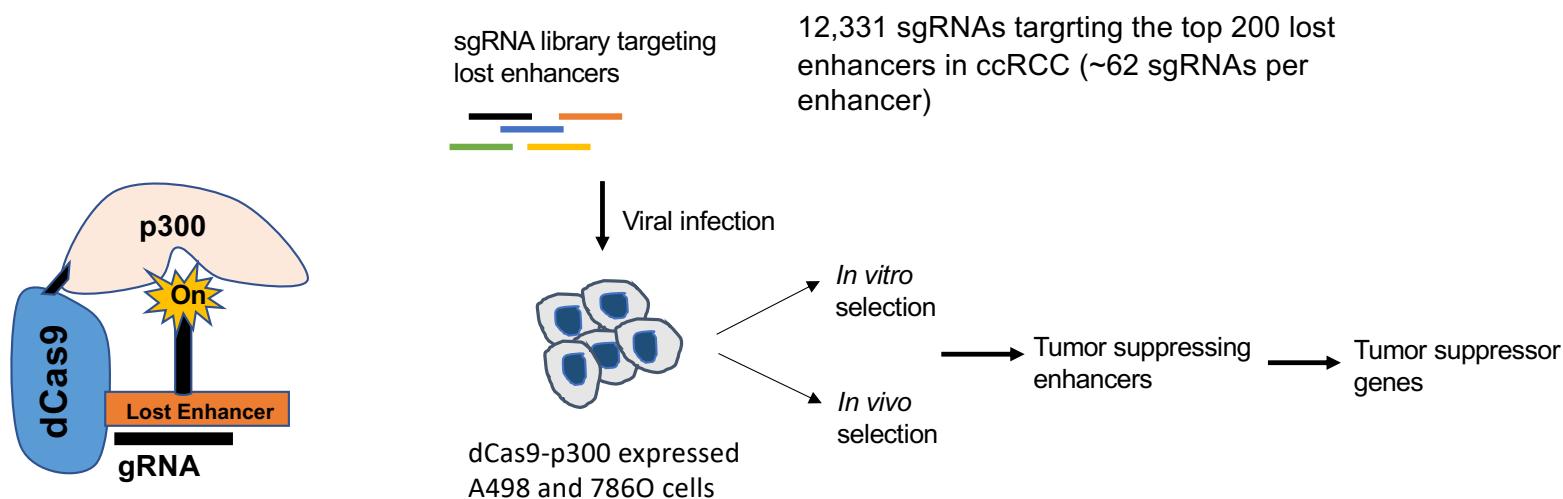
Plans:

1. Select 2 individual sh-RNAs for each gene to test in more cell lines (A498, UMRC-2, UMRC-6, CAKi-1 and HKC) in 2D and 3D assay.
2. Using Dox-inducible sgRNA knockout of the three genes in 786-O and A498 cells, and perform in vivo tumor study (Subcutaneous tumor first, then orthotopic)
3. H3K27ac-HiChIP assay to map the genomic interaction with active enhancer regions in 786-O cells
4. Exam the enhancer regulations of the three candidate gene expressions by sgRNA mediated deletion of the enhancer region.

## Project 2: Identification of tumor suppressor genes through functional screening of lost enhancers in ccRCC

Hypothesis: Reactivate the *vhl*-mediated lost enhancers would identify new tumor suppressor genes in ccRCC

Strategy: Using dCas9-p300 gene activation system targeting the lost enhancer to screen for potential tumor suppressors



## Establishing dCas9-p300 cell lines

