

Supplementary Materials

Resistance to MET/VEGFR2 inhibition by cabozantinib is mediated by YAP/TBX5-dependent induction of FGFR1 in castration-resistant prostate cancer

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Table S1. shRNA sequences

Met1 shRNA#1 target	GTAAAGAGGCACTAGCAAA
Non-Target control shRNA	TTCTCCGAACGTGTCACGT
TBX5 shRNA#1 target	GCCGACGATCACAGATACAAA
TBX5 shRNA#2 target	GCTGCACAGAATGTCAAGAAT
YAP shRNA#1 target	GCCACCAAGCTAGATAAAGAA
YAP shRNA#2 target	CAGGTGATACTATCAACCAAA
Non-target Control shRNA (RFP)	SIGMA-ALDRICH, Lot 03241711MN
PCDNA3.1-FGFR1+P2A-eGFP	GeneScript, Clone ID NM_023110.2

Table S2. PCR primers

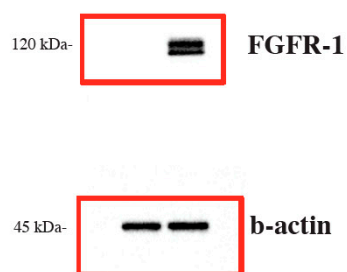
GAPDH	Forward Primer	5'-CCTGCACCACCAACTGCTTA-3'
	Reverse Primer	5'-GGCCATCCACAGTCTTCTGAG-3'
FGF1	Forward Primer	5'-GGGCTTTTATACGGCTACA-3'
	Reverse Primer	5'-TGCTTCTGGATATATAGGTGTTGTAA-3'
FGF2	Forward Primer	5'-AGAAGAGCGACCCTCACATCA-3'
	Reverse Primer	5'-CGGTTAGCACACACTCCTTTG-3'
TBX5	Forward Primer	5'-GGGCTCCAGTACCAGTGTGA-3'
	Reverse Primer	5'-GTAGGGCTTCTGTAGGGATG-3'
GINS1	Forward Primer	5'-CCCCAAAAGTGTCCATGACC-3'
	Reverse Primer	5'-CAGGATCACCCCATCTCAA-3'
RRM2	Forward Primer	5'-CTGTTTCTATGGCTTCCAAAT-3'
	Reverse Primer	5'-TTCTTCTTACACAAGGCATT-3'
YAP	Forward Primer	5'-CGCTCTTCAACGCCGTC-3'
	Reverse Primer	5'-AGTACTGGCCTGTCCGGAGT-3'

Table S3. Antibodies used in the study.

Antigen	Dilution	Catalogue No	Source
Immunohistochemistry			
FGFR-1	1:300	NB100-2080	Novus
TBX5	1:500	PA5-29845	Invitrogen
YAP	1:500	12395	Cell signaling
CD31	1:100	77699	Cell signaling
Immunofluorescence			
TBX5 (A-6)	1:100	sc-515536	Santa Cruz
YAP	1:250	NBP110-58358	Novus Biologicals, CO
Immunoblotting			
MET (D1C2)	1:1000	8198	Cell Signaling

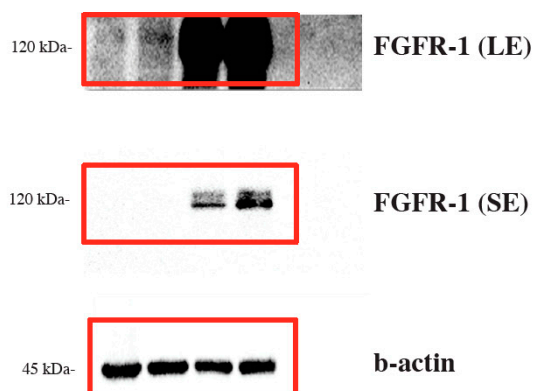
p-MET (Y1234/1235)	1:1000	3077	Cell Signaling
YAP (1A12)	1:1000	12395	Cell Signaling
p-YAP (S127) (D9W2I)	1:1000	13008	Cell Signaling
FGFR-1 (D8E4)	1:1000	9740	Cell Signaling
p-FGFR-1 (T766) (1E5)	1:1000	2544	Cell Signaling
TBX5 (A-6)	1:1000	sc-515536	Santa Cruz
β -actin (13E5)	1:1000	4970	Cell Signaling
Vinculin	1:10000	V9131	Sigma-Aldrich, St. Luis, MO, USA
AR (N-20)	1:1000	sc-816	Santa Cruz
p-LATS1 (S909)	1:1000	9157	Cell Signaling
LATS1 (C66B5)	1:1000	3477	Cell Signaling
MST1	1:1000	3682	Cell Signaling
p-MST1/2	1:1000	3681	Cell Signaling

Figure 1D



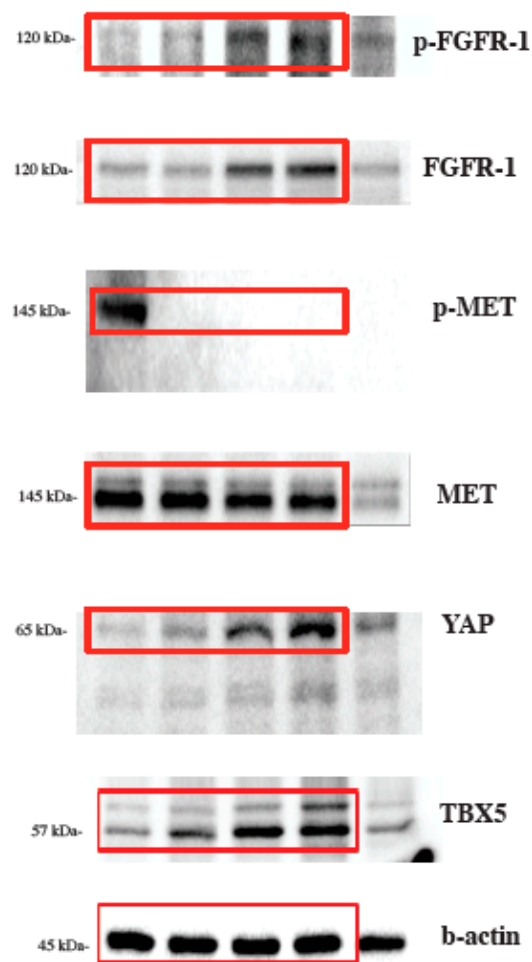
Sample	NT	OV	
Density Ratio	1.00	10.23	FGFR-1

Figure 1G



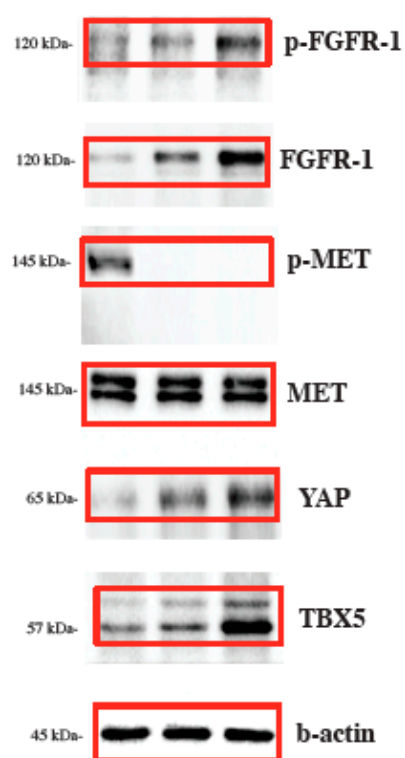
Sample	NT	NT tr.	OV	OV tr.	
Density Ratio	0.00	0.00	3.35	6.46	FGFR-1 (SE)

Figure 2C



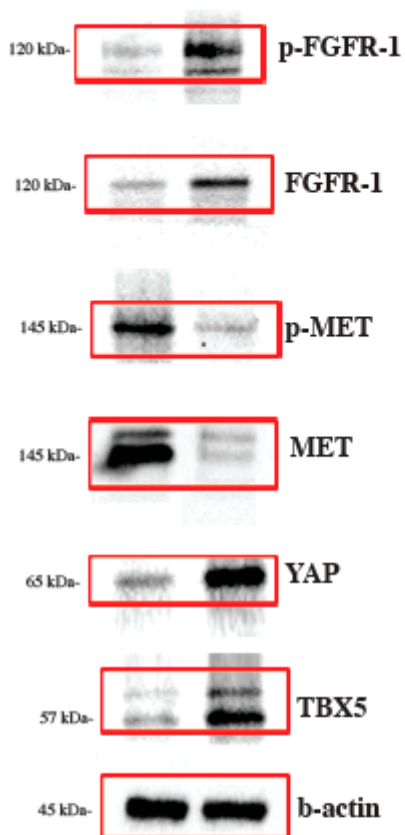
Sample	Cabo (1μM)				Days	
	0	14	28	42		
Density Ratio	1.00	1.89	6.59	8.47		p-FGFR-1
	1.00	1.08	3.06	4.32		FGFR-1
	1.00	0.00	0.00	0.00		p-MET
	1.00	0.86	0.78	0.80		MET
	1.00	2.20	4.32	9.33		YAP
	1.00	3.12	4.84	7.31		TBX5

Figure 2D



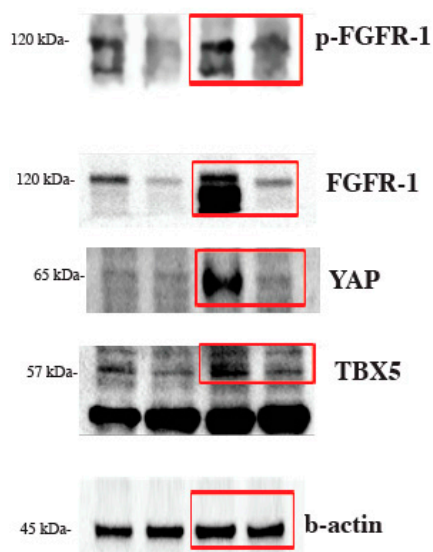
Sample	0	0.8	1	Cabo (1μM)
Density Ratio	1.00	1.55	4.05	p-FGFR-1
	1.00	3.25	5.12	FGFR-1
	1.00	0.00	0.00	p-MET
	1.00	1.03	0.99	MET
	1.00	5.11	7.83	YAP
	1.00	2.35	7.19	TBX5

Figure 2E



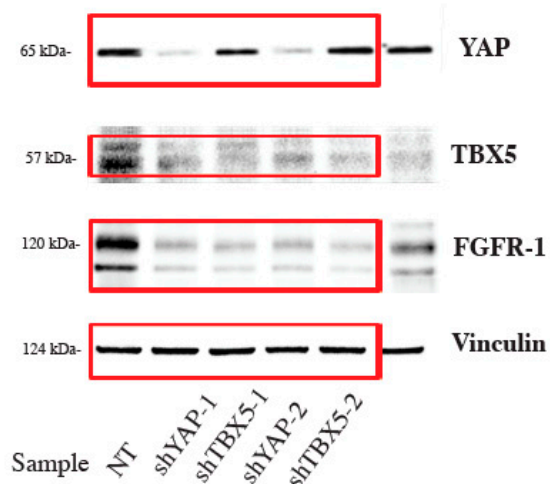
Sample	NT	shMET	
Density Ratio	1.00	5.49	p-FGFR-1
	1.00	5.71	FGFR-1
	1.00	0.27	p-MET
	1.00	0.18	MET
	1.00	9.81	YAP
	1.00	8.12	TBX5

Figure 3A

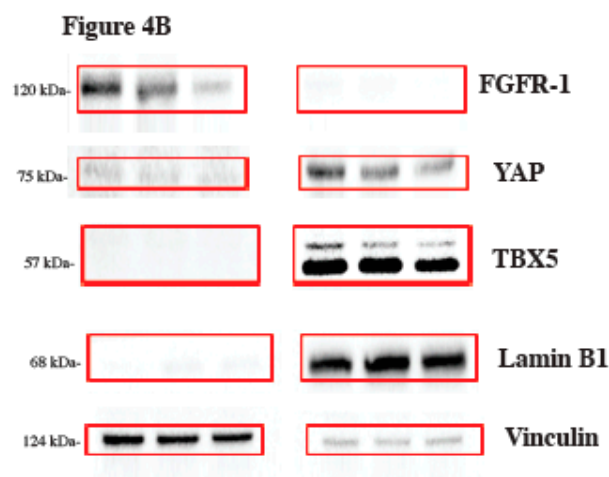


Sample	PC3	144-13	
Density Ratio	1.00	0.35	p-FGFR-1
	1.00	0.11	FGFR-1
	1.00	0.17	YAP
	1.00	0.35	TBX5

Figure 3D

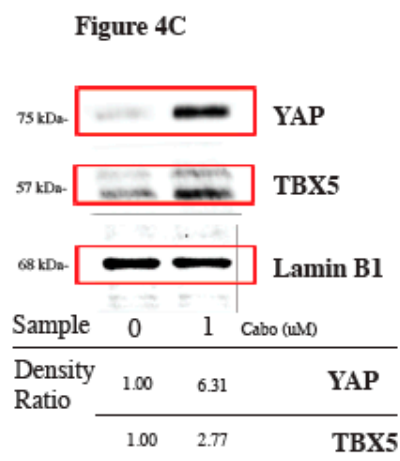


Sample	NT	shYAP-1	shTBX5-1	shYAP-2	shTBX5-2	
Density Ratio	1.00	0.10	0.85	0.18	0.90	YAP
	1.00	0.23	0.18	0.22	0.06	TBX5
	1.00	0.18	0.13	0.19	0.10	FGFR-1



	Cytoplasm			Nucleus			
Sample	0	24	48	0	24	48	
Density Ratio	1.00	0.83	0.27	0.05	0.02	0.01	FGFR-1
	0.15	0.09	0.08	1.15	0.77	0.69	YAP
	0.22	0.17	0.10	1.15	0.84	0.81	TBX5

*Cytoplasmic extracts were normalized to vinculin, whereas nuclear to Lamin B1.



Sample	0	1	Cabo (uM)
Density Ratio	1.00	6.31	YAP
	1.00	2.77	TBX5

Figure 4D

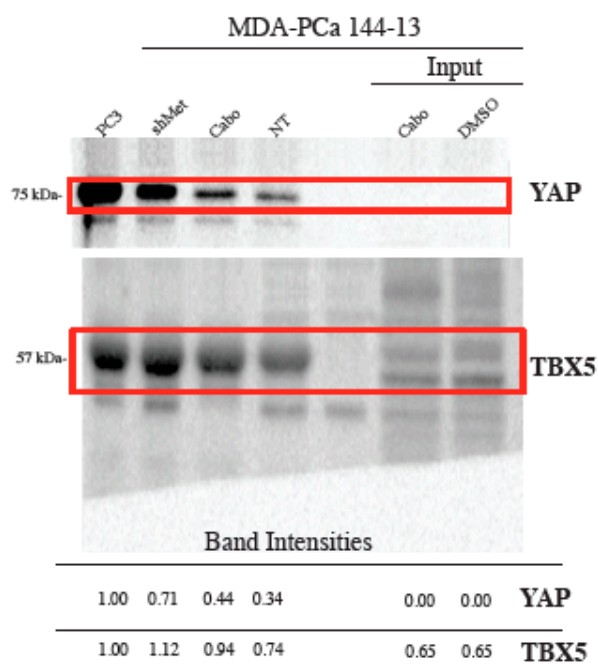
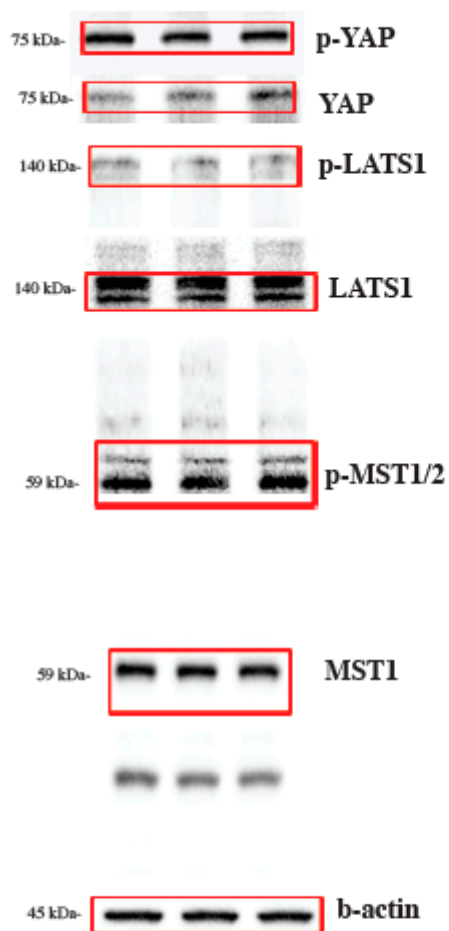
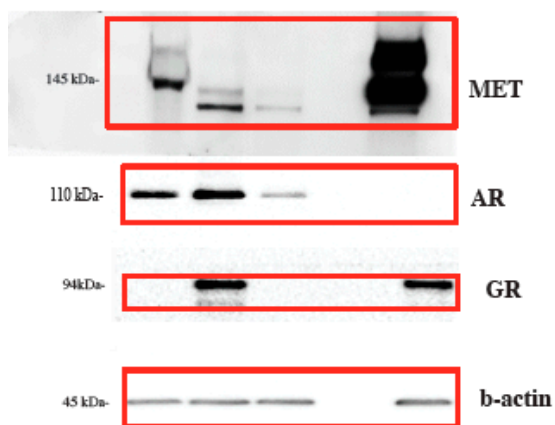


Figure 4E



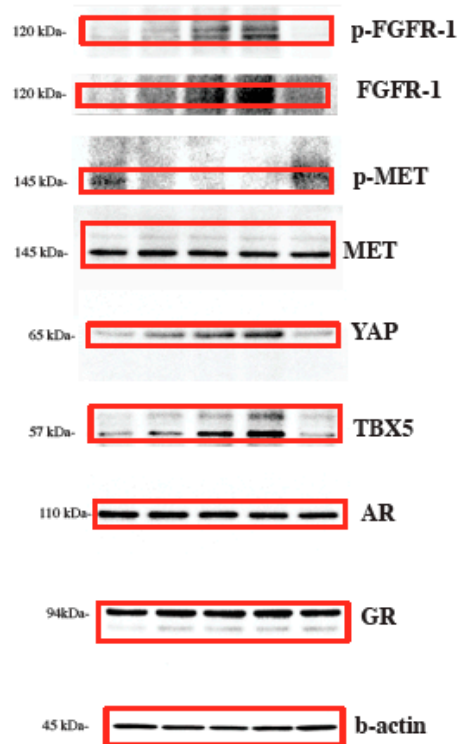
Sample	0	0.8	1	Cabo (uM)
Density Ratio	1.00	0.91	0.98	p-YAP
	1.00	1.37	2.09	YAP
	1.00	1.30	1.27	p-LATS1
	1.00	0.93	1.02	LATS1
	1.00	0.96	1.10	p-MST1/2
	1.00	1.00	0.99	MST1/2

Figure 5A



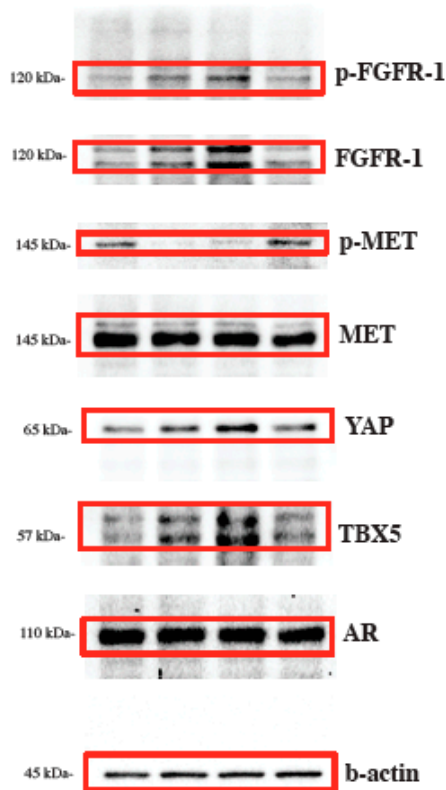
Sample	903L	LREX	LNCaP	PC3	
Density Ratio	9.75	6.61	3.33	19.49	MET
	3.25	4.74	1.10	0.00	AR
	0.01	3.77	0.00	3.11	GR

Figure 5B



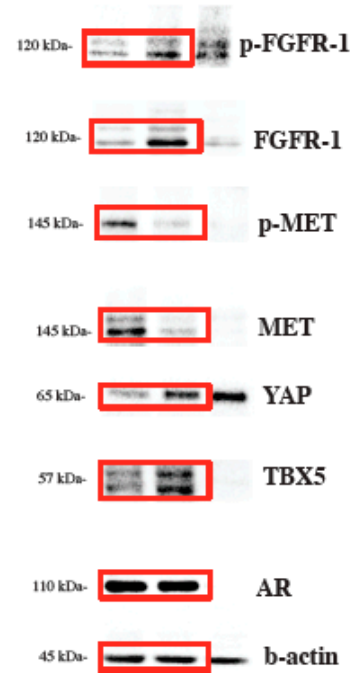
Sample	Cabo (1uM)					DMSO	Days	
	0	14	21	28	28			
Density Ratio	1.00	1.83	4.45	7.57	0.74			p-FGFR-1
	1.00	3.20	7.66	12.13	2.14			FGFR-1
	1.00	0.18	0.14	0.06	1.12			p-MET
	1.00	1.34	1.48	1.17	1.02			MET
	1.00	3.12	5.73	7.18	1.02			YAP
	1.00	2.81	8.16	12.56	1.02			TBX5
	1.00	1.14	1.26	1.00	1.84			AR
	1.00	1.33	1.59	1.36	1.04			GR

Figure 5C



LNCaP					
Sample	Cabo (1uM)			DMSO	Days
	0	14	21	21	
Density Ratio	1.00	1.83	3.20	0.89	p-FGFR-1
	1.00	3.90	7.78	1.00	FGFR-1
	1.00	0.04	0.27	0.95	p-MET
	1.00	0.80	0.82	0.73	MET
	1.00	2.85	7.32	1.10	YAP
	1.00	2.63	5.82	1.00	TBX5
	1.00	0.85	0.86	0.74	AR

Figure 5D



Sample	LREX		
	NT	shMet	
Density Ratio	1.00	3.54	p-FGFR-1
	1.00	5.64	FGFR-1
	1.00	0.10	p-MET
	1.00	0.20	MET
	1.00	3.24	YAP
	1.00	4.83	TBX5
	1.00	0.96	AR

