

Supplementary Material

BTK Isoforms p80 and p65 Are Expressed in Head and Neck Squamous Cell Carcinoma (HNSCC) and Involved in Tumor Progression

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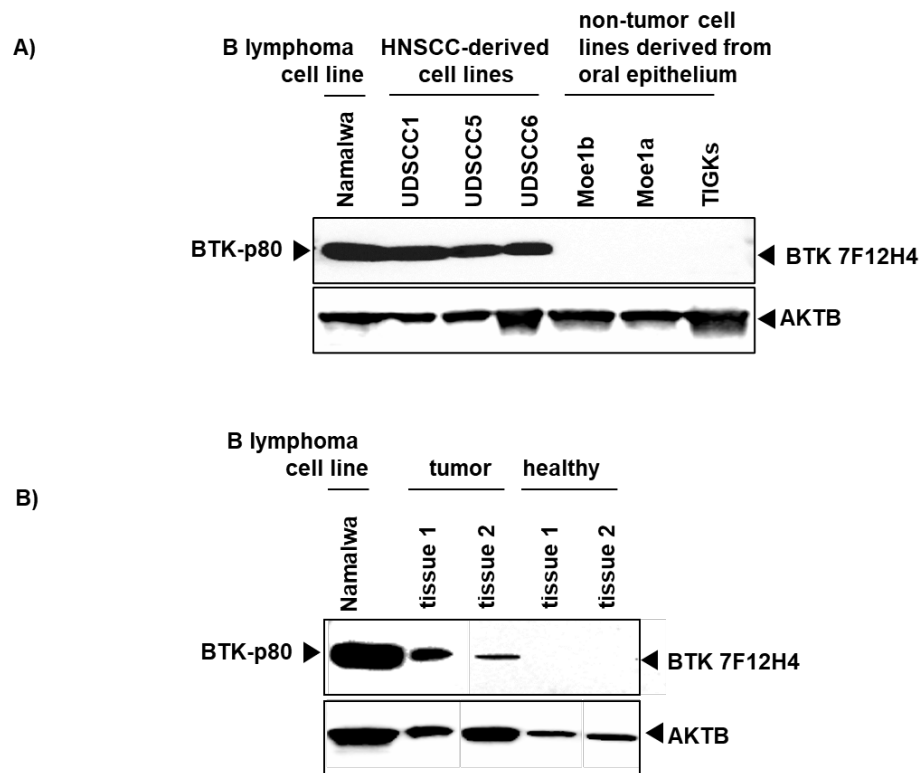
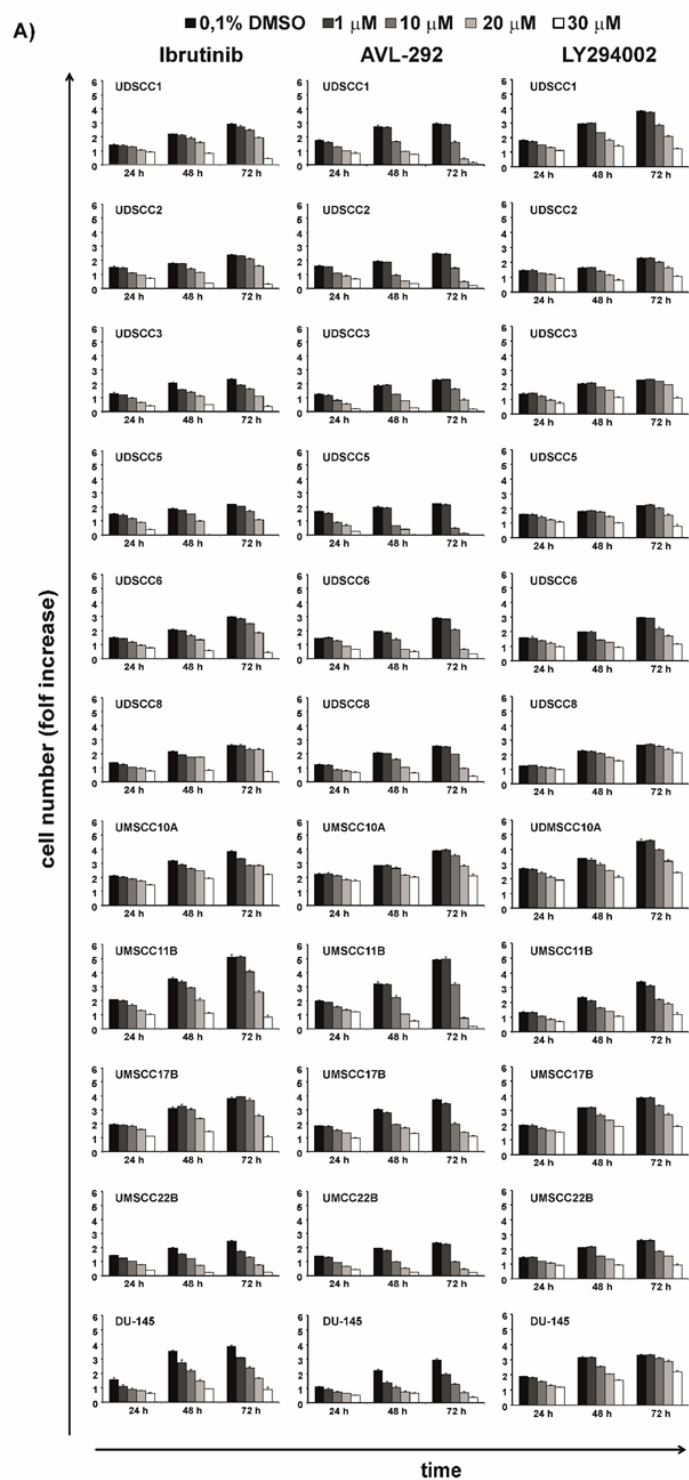


Figure S1: (A) Expression of BTK-p80 isoform was determined in one B cell lymphoma cell line (Namalwa), three HNSCC-derived cell lines (UDSCC1, UDSCC5, UDSCC6) as well as in non-tumor cell lines derived from oral epithelium (Moe1a, Moe1b, TIGK) by immunoblotting using BTK antibody 7F12H4. (B) Expression of BTK-p80 was determined in primary cancer tissue specimens of the head and neck region and in healthy non-tumor squamous epithelial cells obtained from the same individuals using BTK antibody 7F12H4. β -actin was used as loading control.



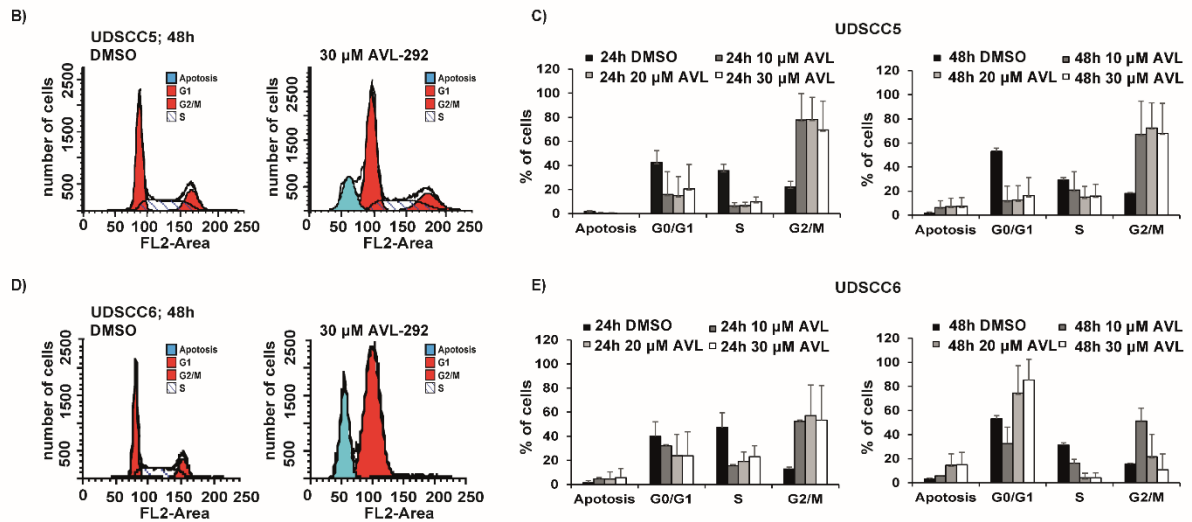
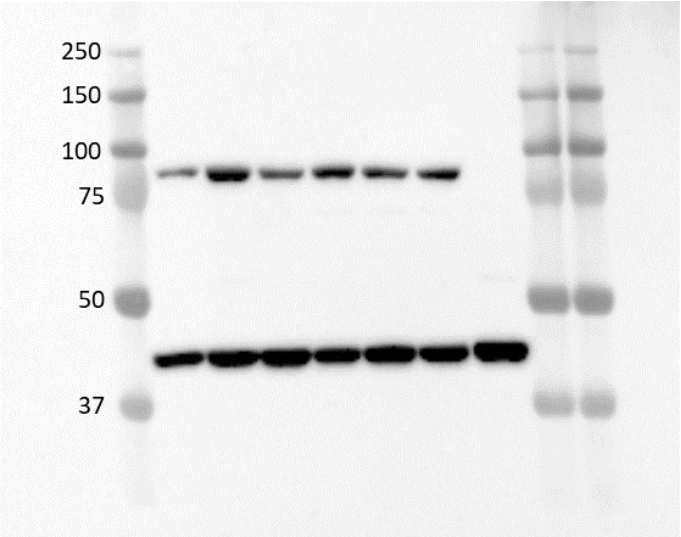
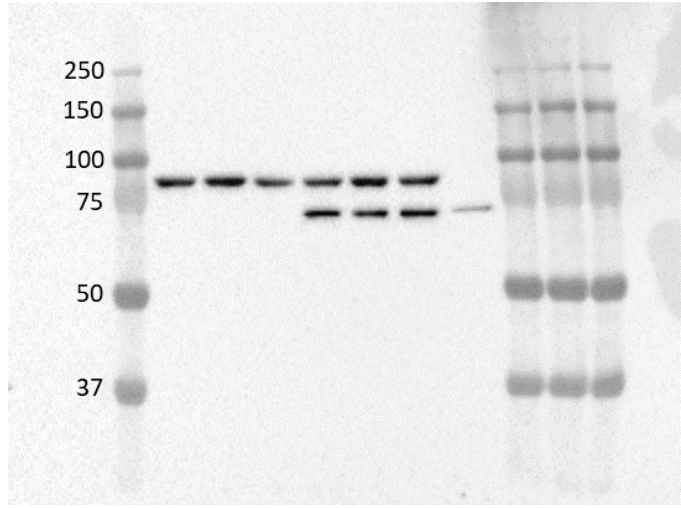
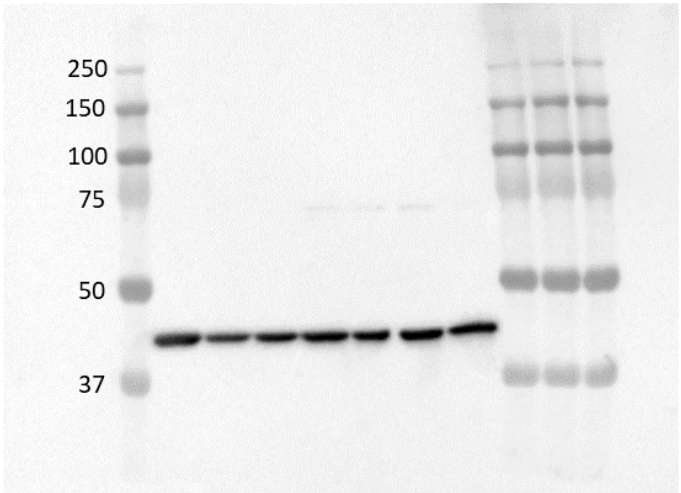

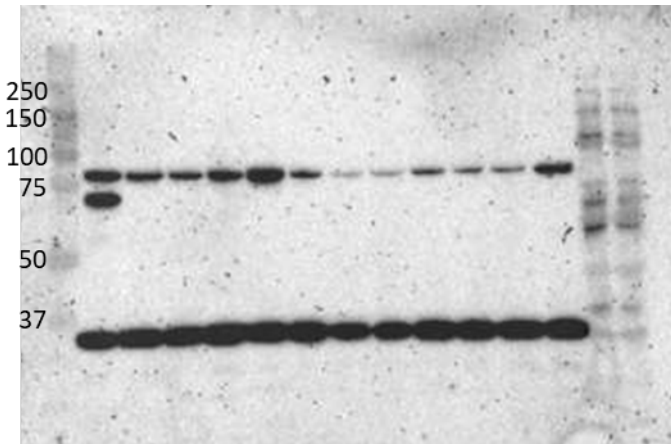



Figure S2: BTK inhibition impairs proliferation and induces cell cycle arrest and apoptosis. (A) BTK inhibition decreased proliferation of HNSCC-derived cell lines. Ten HNSCC-derived cell lines and the prostate carcinoma cell line DU-145 were seeded and treated with different concentrations (1, 10, 20 and 30 μ M) of three different BTK inhibitors (Ibrutinib, AVL-292 and LY294002). Treatment with 0.1 % DMSO served as control. Proliferation was assessed over a time. This experiment was performed in triplicates. Presented are the mean values. (B-E) BTK inhibition induces cell cycle arrest and apoptosis. Cell cycle and apoptosis were analyzed using UDSCC5 (B, C) or UDSCC6 (D, E) cells that were either left untreated (DMSO control) or treated with different concentrations (10, 20 or 30 μ M) of AVL-292. Cell cycle and apoptosis was assessed 24h and 48h after treatment. Representative graphs of cell cycle analyses are shown in D and F. Depicted in E and G are mean values \pm SD of three independent experiments.

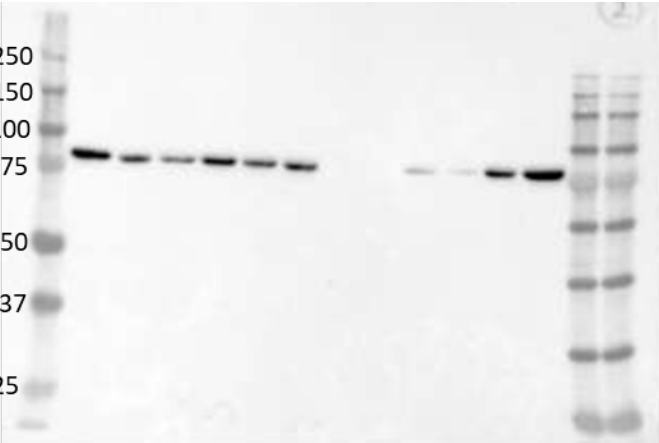
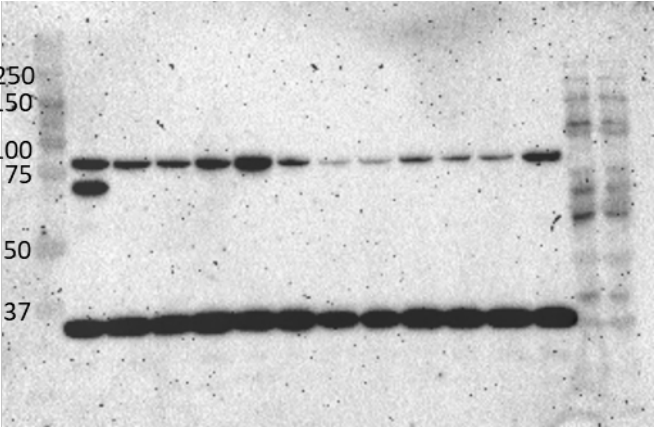
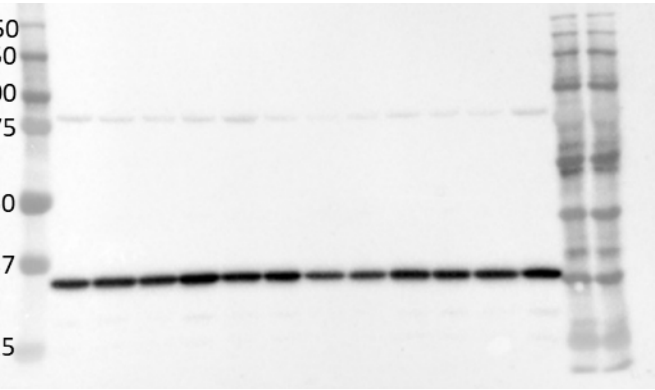
Original blots of Figure 1F

	<p>BTK 7F12H4 (80 kDa)</p> <p>β-actin (42 kDa)</p>	<p>Lane 1: Marker Lane 2: UD-SCC1 Lane 3: UD-SCC5 Lane 4: UD-SCC6 Lane 5: Ramos Lane 6: Namalwa Lane 7: Preb1 Lane 8: CD19+ cells Lane 9: Marker Lane 10: Marker</p>
	<p>BTK DFS (80 and 77 kDa)</p> <p>β-actin (42 kDa)</p>	
	<p>β-actin (42 kDa)</p>	

Original blots of Figure 1G (right panel)

	BTK 7F12H4 (80 kDa)	Lane 1: Marker Lane 2: Namalwa Lane 3: UD-SCC1 Lane 4: UD-SCC2 Lane 5: UD-SCC3 Lane 6: UD-SCC5 Lane 7: UD-SCC6 Lane 8: UD-SCC8 Lane 9: UD-SCC10A Lane 10: UM-SCC11B Lane 11:UM-SCC17B Lane 12:UM-SCC22B Lane 13: Marker Lane 14: Marker
	BTK DFS (80 and 77 kDa)	
	GAPDH (37 kDa)	

Original blots of Figure 1G (left panel)

	BTK 7F12H4 (80 kDa)	Lane 1: Marker Lane 2: Namalwa Lane 3: not of interest Lane 4: not of interest Lane 5: DU-145 Lane 6: LNCaP Lane 7: PC-3 Lane 8: not of interest Lane 9: not of interest Lane 10: not of interest Lane 11: not of interest Lane 12: not of interest Lane 13: Marker Lane 14: Marker
	BTK DFS (80 and 77 kDa)	
	GAPDH (37 kDa)	

Original blots of Figure 1H

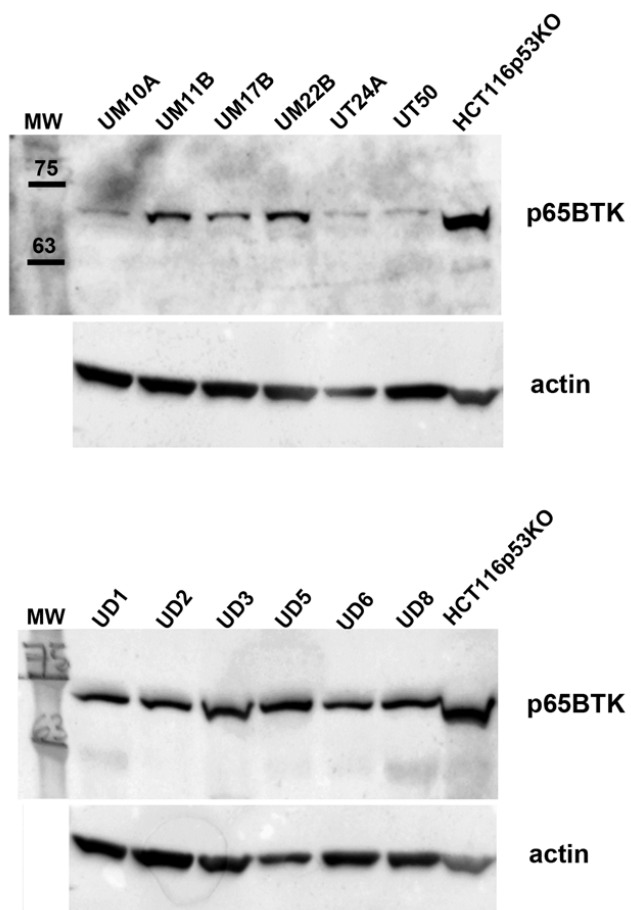
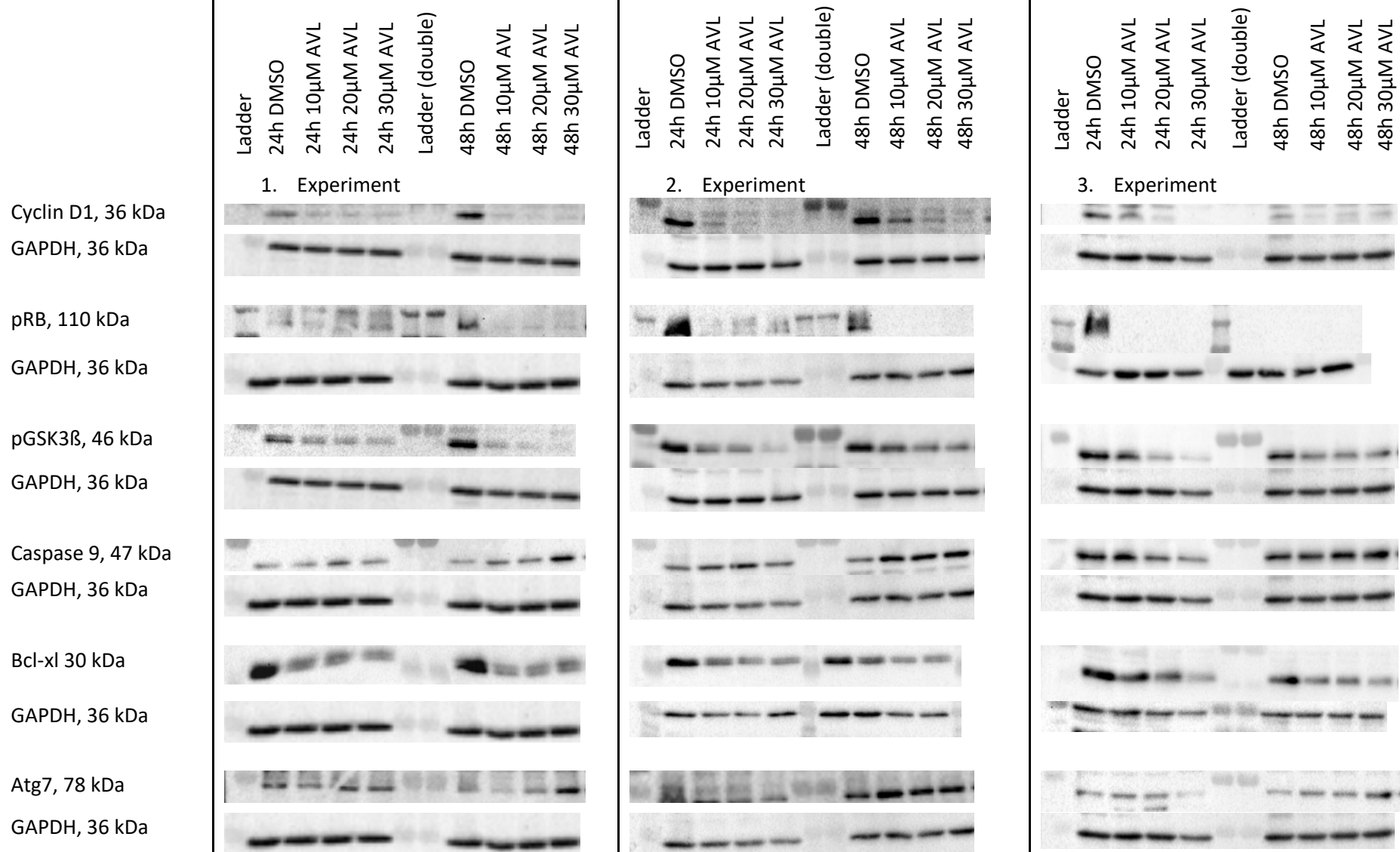


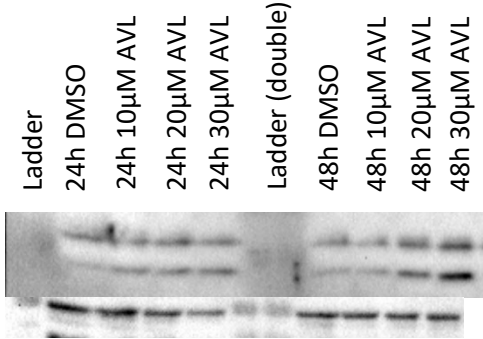
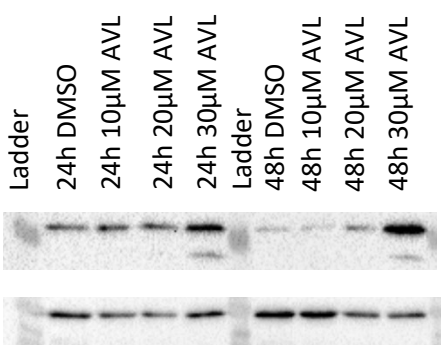
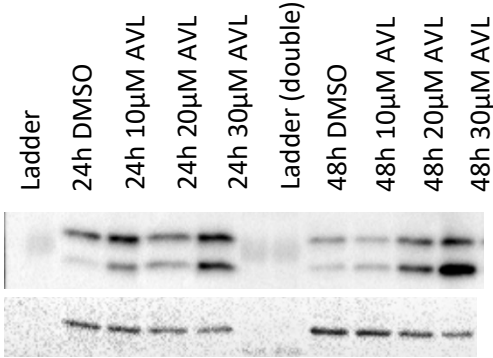
Figure S3: Original membranes of immunoblots shown in Figure 1 F-H.

UD-SCC1

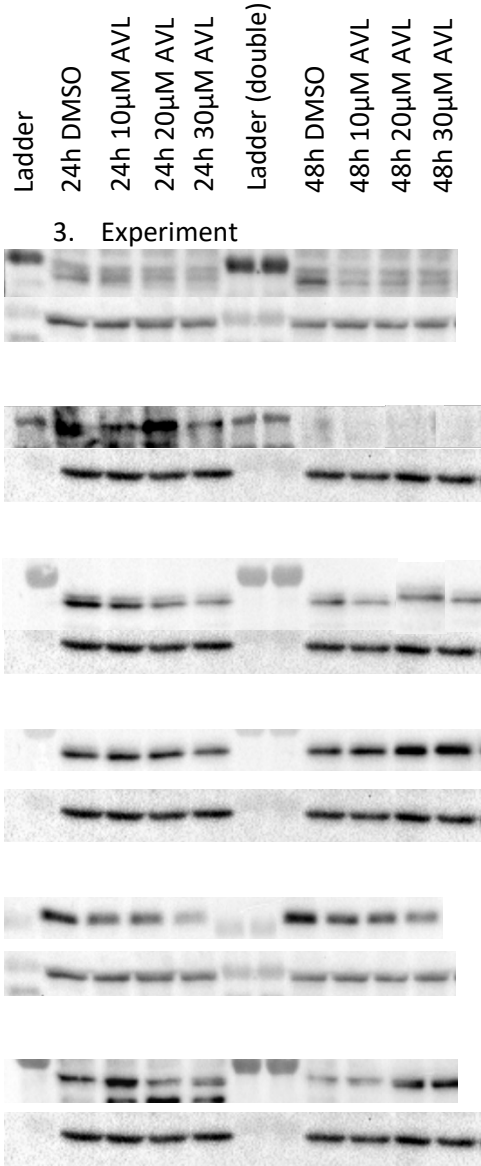
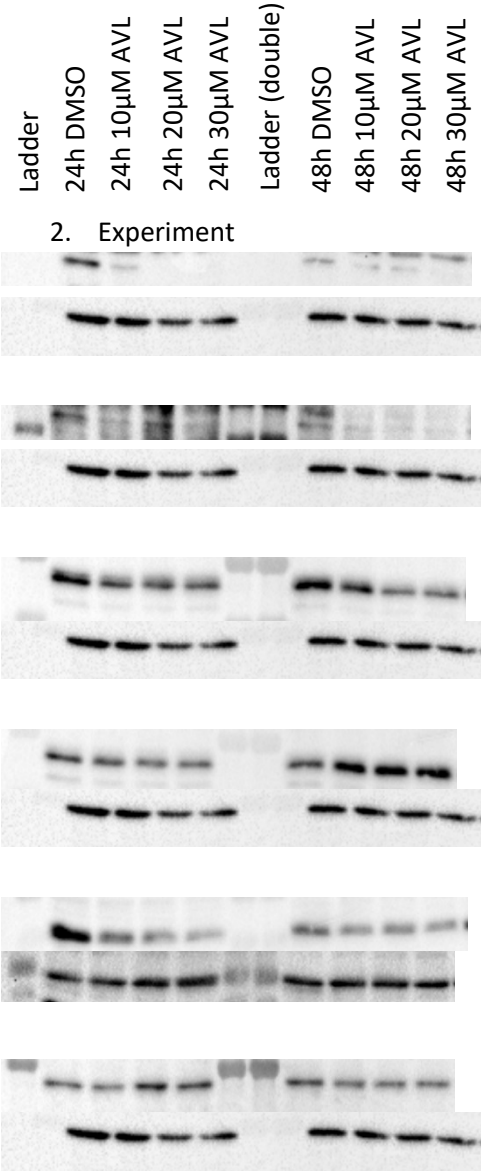
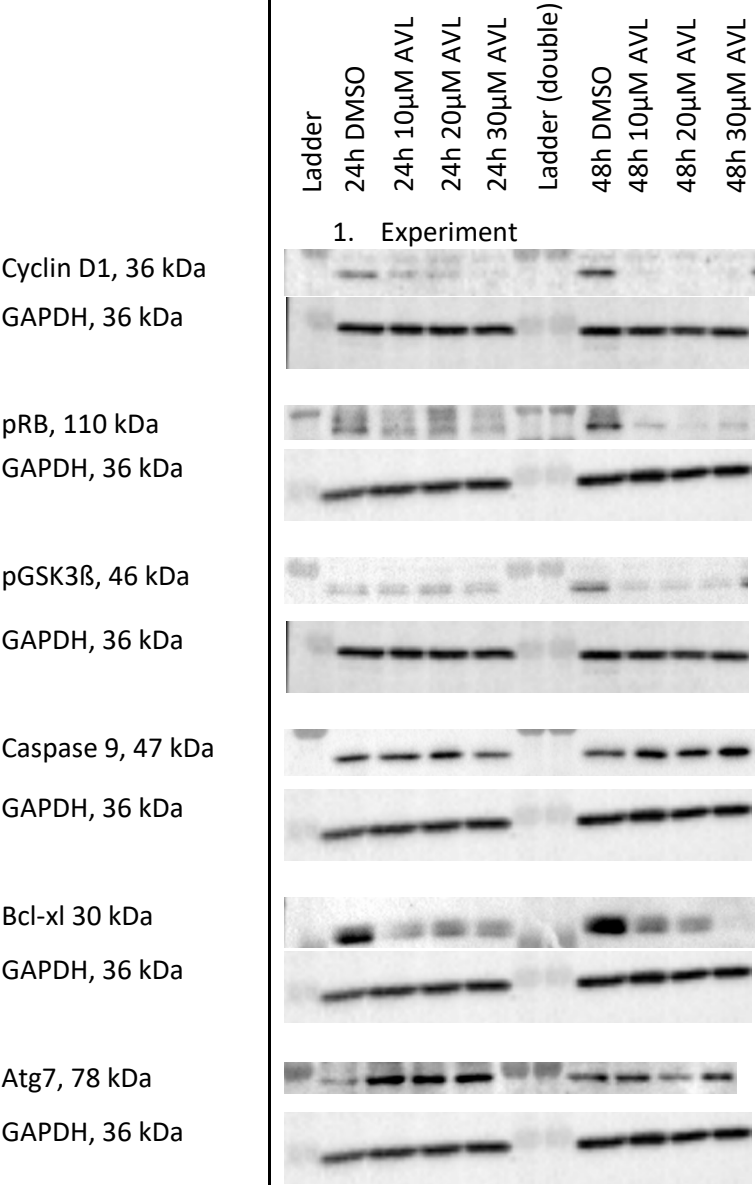


UD-SCC1

LC3A/B I, 17 kDa
LC3A/B II, 14 kDa
GAPDH, 36 kDa

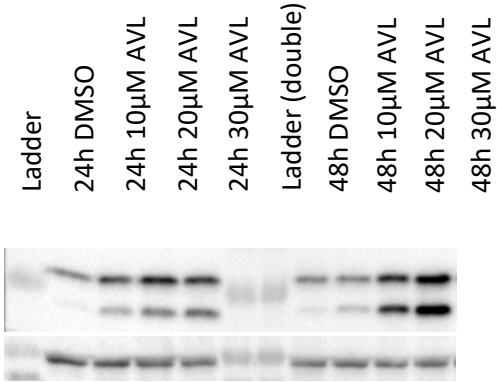
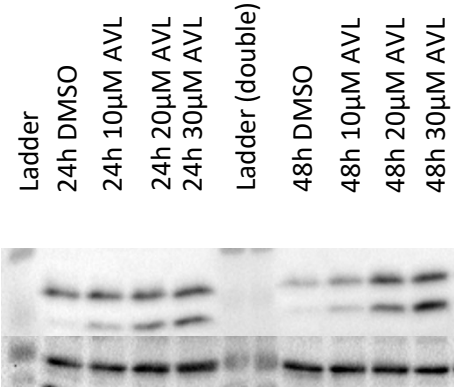
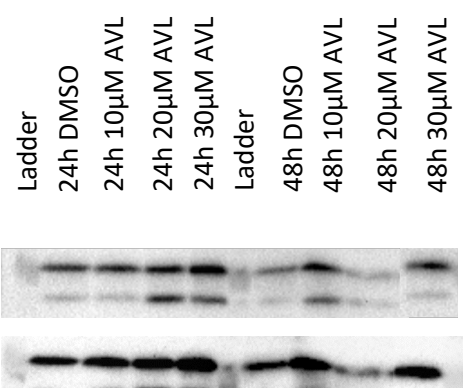


UD-SCC 2

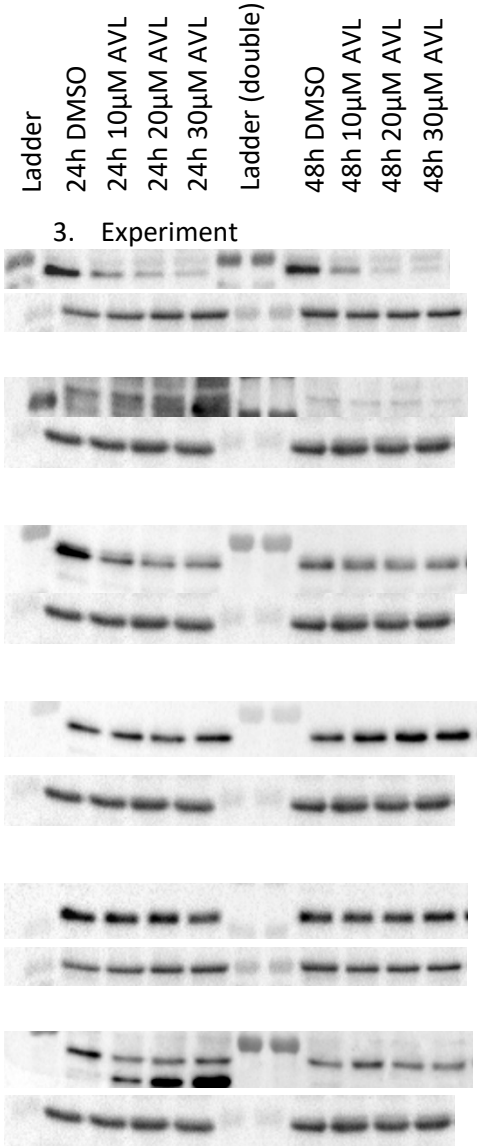
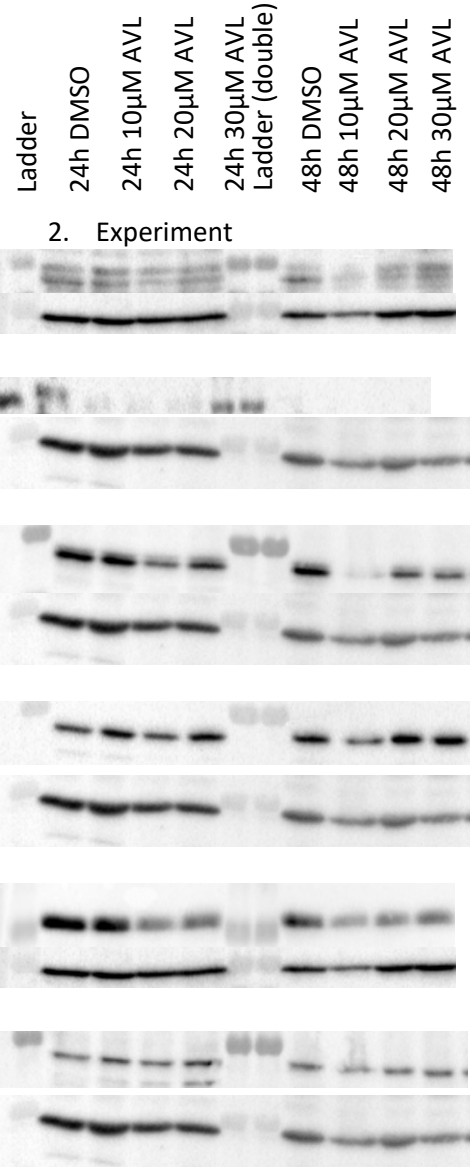
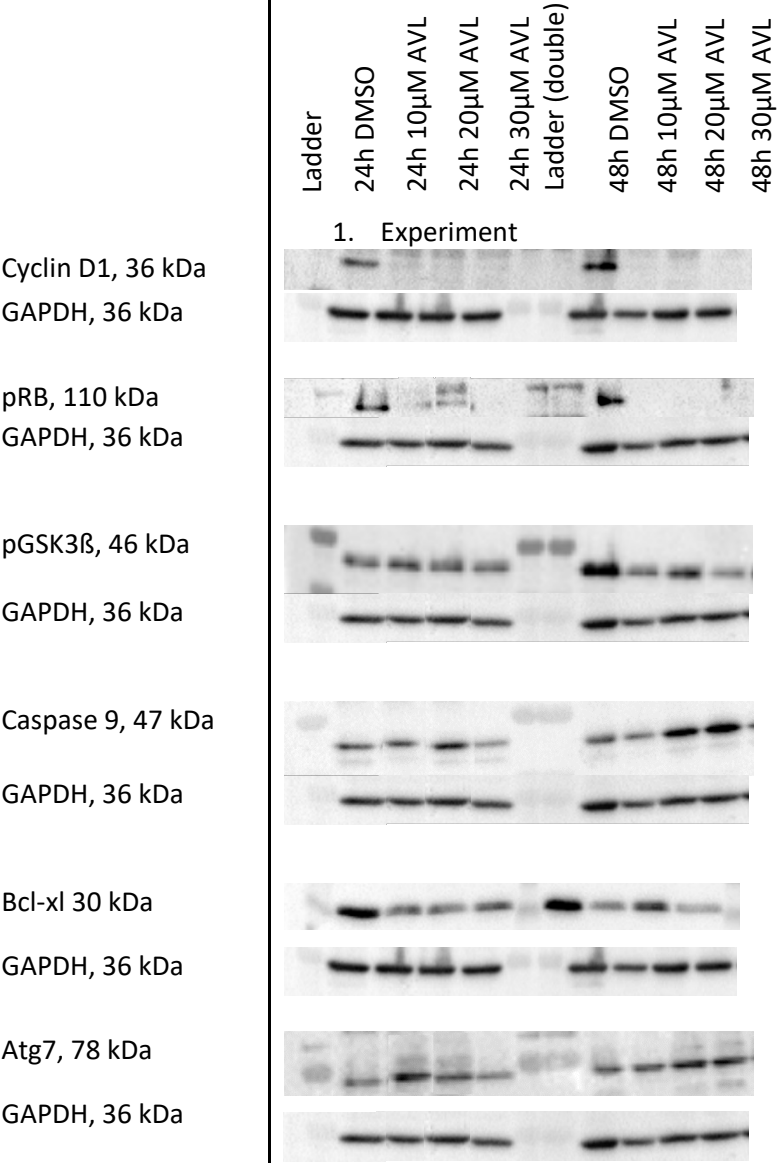


UD-SCC 2

LC3A/B I, 17 kDa
LC3A/B II, 14 kDa
GAPDH, 36 kDa



UD-SCC 6



UD-SCC 6

LC3A/B I, 17 kDa
LC3A/B II, 14 kDa
GAPDH, 36 kDa

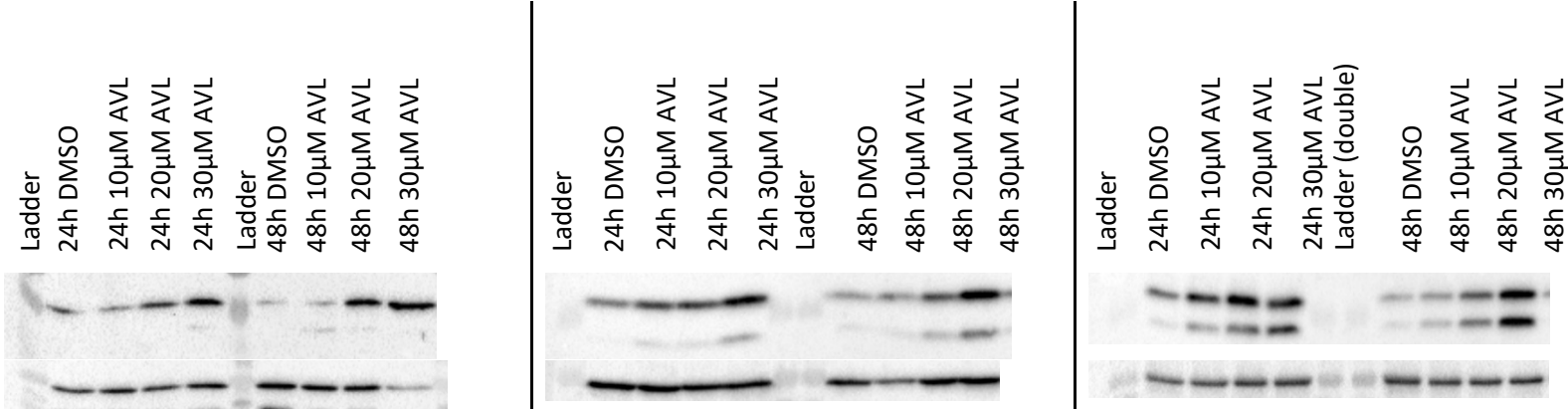


Figure S4: Original membranes of immunoblots shown in Figure 3.

Original blots of Figure 5A

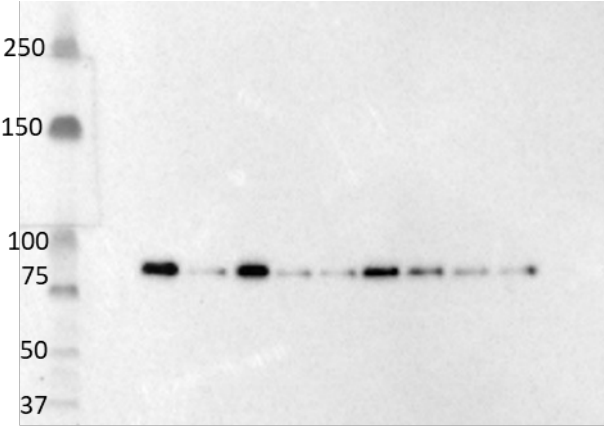
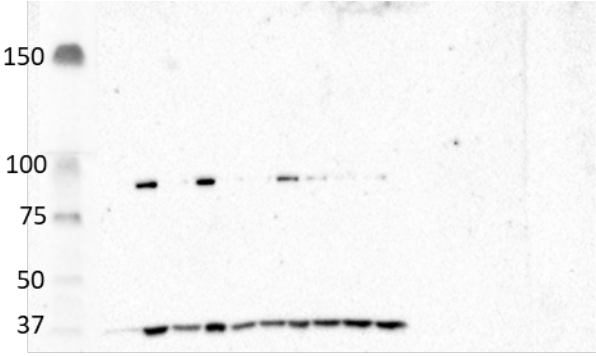
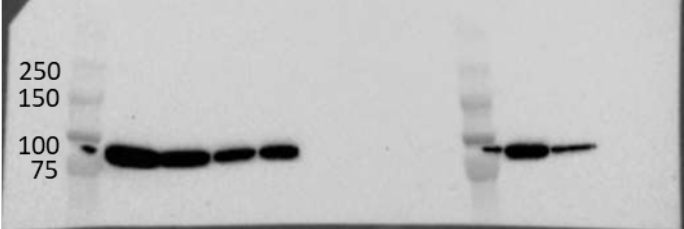
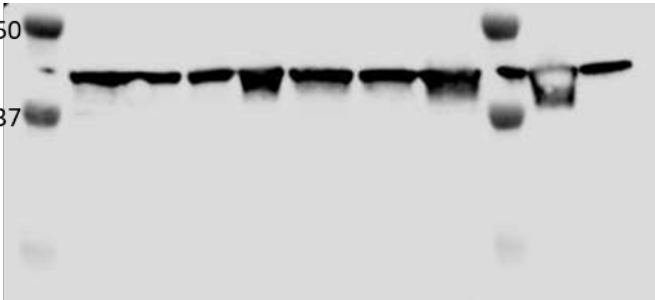
	<p>BTK 7F12H4 (80 kDa)</p>	<p>Lane 1: Marker Lane 2: Scr 1 Lane 3: BTK 1 Lane 4: Scr OZ 1 Lane 5: BTK OZ 1 Lane 6: BTK PB 1 Lane 7: Scr OZ 5 Lane 8: BTK OZ 5 Lane 9: Scr PB 6 Lane 10: BTK PB 6</p>
	<p>GAPDH (37 kDa)</p>	

Figure S5: Original membranes of immunoblots shown in Figure 5A.

Original blots of Figure S1A

	BTK 7F12H4 (80 kDa)	Lane 1: Marker Lane 2: Namalwa Lane 3: UDSCC1 Lane 4: UDSCC5 Lane 5: UDSCC6 Lane 6: Moe1b Lane 7: Moe1a Lane 8: TIGKs Lane 9: marker Lane 10: not of interest Lane 11: not of interest
	β-actin (42 kDa)	

Original blots of Figure S1B

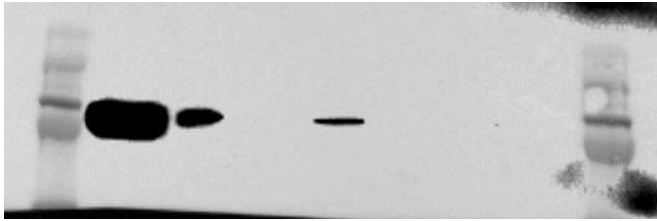
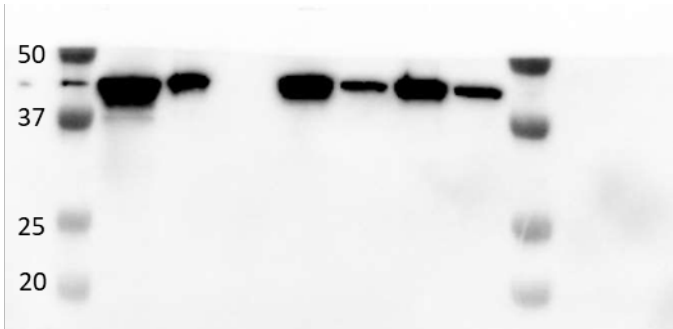
	<p>BTK 7F12H4 (80 kDa)</p>	<p>Lane 1: Marker Lane 2: Namalwa Lane 3: tumor tissue 1 Lane 4: tumor tissue 3 Lane 5: tumor tissue 2 Lane 6: healthy tissue 1 Lane 7: healthy tissue 3 Lane 8: healthy tissue 2 Lane 9: marker</p>
	<p>β-actin (42 kDa)</p>	

Figure S6: Original membranes of immunoblots shown in Figure S1.

Table S1: HNSCC cell lines

Cell line	Sex	Site of origin	TNM stage	HPV 16 status	TP53 gene mutation
UDSCC1	M	Oropharynx	T3N2bM0	-	Skip exon 3
UDSCC2	M	Hypopharynx	T1N2M0	+	wt
UDSCC3	M	Larynx	T2N2cM0	-	Nonsense, p.224Glu->Stop
UDSCC5	M	Larynx	T1N1M0	-	Missense, p.179His->Tyr
UDSCC6	M	Oropharynx	T2N0M0	-	Missense, p.220Tyr->Cys
UDSCC8	/	Larynx	/	-	Missense, p.155Thr->Asn
UMSCC10A	M	Larynx	T3N0M0	-	Missense, p.245Gly->Cys
UMSCC11B	M	Larynx	T2N2aM0	-	Missense, p.242Cys->Ser
UMSCC17B	F	Larynx	T1N0M0	-	wt
UMSCC22B	F	Hypopharynx	T2N1M0	-	Missense, p.220Tyr->Cys

Table S2: Non-tumor oral epithelial cell lines

Cell line	Sex	Site of origin	Reference
Moe1a	unspecified	Oral epithelium	[27]
Moe1b	unspecified	Oral epithelium	[27]
hTERT TIGKs	M	Gingiva; Epithelium	[28]

Table S3: Clinical data of patient samples

Patient	Sex	Site of origin	TNM stage	HPV status
1	M	Oral cavity	T2N0M0	n.a.
2	M	Oral cavity	T2N0M0	n.a.