

Ci-VSP	MEGFDGSDFSPPADLVGVDGAVMRNVV-DVTINGD-VTAPPKAAAPRKSESVKKVHWNDVDQGPSEKPTRQE	70
Hs-VSP1	-----MNESPQTNEFKGTTEEAPAKESPHTSEFKGAALV-----SPISK-----	39
Hs-VSP1 _{CiV}	MEGFDGSDFSPPADLVGVDGAVMRNVV-DVTINGD-VTAPPKAAAPRKSESVKKVHWNDVDQGPSEKPTRQE	70
PTEN _{CiV}	MEGFDGSDFSPPADLVGVDGAVMRNVV-DVTINGD-VTAPPKAAAPRKSESVKKVHWNDVDQGPSEKPTRQE	70
PTEN	-----	
Ci-VSP	ERIDIPEISGLWWGENEHGVDDGRMEIPTTGVRVQFRVRAVIDHLGMRVFGVFLIFLDIILMIIDLSPGK	142
Hs-VSP1	-----SMLERLSKFEVEDA--ENVASYDSKIKKIVHSIVSSFAFGIFGVFLVLLDVTLLADLIFTDS	100
Hs-VSP1 _{CiV}	ERIDIPEISGLWWGENEHGVDDGRMEIPTTGVRVQFRVRAVIDHLGMRVFGVFLIFLDIILMIIDLSPGK	142
PTEN _{CiV}	ERIDIPEISGLWWGENEHGVDDGRMEIPTTGVRVQFRVRAVIDHLGMRVFGVFLIFLDIILMIIDLSPGK	142
PTEN	-----	
Ci-VSP	SESSQSFYDGMALALSCYFMDLGLRIFAYGPKNFFTNPWEVADGLIIVVTFVVTIFYTVLDEYVQETGADG	214
Hs-VSP1	KLYIPLEYRSISLAIGLFFLMDVLLRVFVEGRQQYFSDLFNILDTAIIVIPLVDVIYIFFDIKLLRN-IPR	171
Hs-VSP1 _{CiV}	SESSQSFYDGMALALSCYFMDLGLRIFAYGPKNFFTNPWEVADGLIIVVTFVVTIFYTVLDEYVQETGADG	214
PTEN _{CiV}	SESSQSFYDGMALALSCYFMDLGLRIFAYGPKNFFTNPWEVADGLIIVVTFVVTIFYTVLDEYVQETGADG	214
PTEN	-----	
Ci-VSP	LGRLVVLARLLRVRLARIFYSHQQMKASSRRTISQNKRRYRKDGFDDLTYVTDHVIAMSFSSGRQSLFR	286
Hs-VSP1	WTHLVRLRLRIILIRIFHLLHQKQLEKLMRRLVSENKRRYTRDGFDDLTYVTERIAMSFPSSGRQSFYR	243
Hs-VSP1 _{CiV}	LGRLVVLARLLRVRLARIFYSHQQLEKLMRRLVSENKRRYTRDGFDDLTYVTERIAMSFPSSGRQSFYR	286
PTEN _{CiV}	LGRLVVLARLLRVRLARIFYSHQQMKASSRRTISQNKRRYQEDGFDDLTYIYPNIIAMGFPAERLEGVYR	286
PTEN	-----MTAIIKEIVSRNKRRYQEDGFDDLTYIYPNIIAMGFPAERLEGVYR	47
Ci-VSP	NPIGEVSFRFFTKHPDKFRIYNLCSERGYDETKFDNHVYRVMIDDDHVPTLVDLLKFIDDAKVVMTSDPDHV	358
Hs-VSP1	NPIEEVVRFLDKKHRNHRYVYNLCSERAYDPKHFNHVRSRIMIDDDHVPTLHEMVVFTKEVNEWMAQDLENI	315
Hs-VSP1 _{CiV}	NPIEEVVRFLDKKHRNHRYVYNLCSERAYDPKHFNHVRSRIMIDDDHVPTLHEMVVFTKEVNEWMAQDLENI	358
PTEN _{CiV}	NNIDDVVRFLDSKHKNHKYIYNLCARHYDTAKFNCRVAQYFFEDHNPQLELIKPFCELDQWLSEDDNHV	358
PTEN	NNIDDVVRFLDSKHKNHKYIYNLCARHYDTAKFNCRVAQYFFEDHNPQLELIKPFCELDQWLSEDDNHV	119
	WPD loop	
Ci-VSP	IAIHCKGKGRTGTTLVSSWLLLEDGKFDTAKEALEYFGSRRTDFEVGDVFQGVETASQIRYVGIFYEKIKKNYG	430
Hs-VSP1	VAIHCKGKGRTGTMVCALLIASEIFLTAESLYYFGERRTNKTHSNKFQGVETPSQNRVYGYFAQVKHLYN	387
Hs-VSP1 _{CiV}	VAIHCKGKGRTGTMVCALLIASEIFLTAESLYYFGERRTNKTHSNKFQGVETPSQNRVYGYFAQVKHLYN	430
PTEN _{CiV}	AAIHCKAGKGRGTGVMICAYLLHRGKFLKAQEALDFYGEVTRTDK----KGVTTIPSQRRYVYYSYLLKNHL	425
PTEN	AAIHCKAGKGRGTGVMICAYLLHRGKFLKAQEALDFYGEVTRTDK----KGVTTIPSQRRYVYYSYLLKNHL	186
	P loop	TI loop
Ci-VSP	GQLPPMKKLKVTGVTTITAIQGVGRNGSDLSMQIVSERQEVLLCKFAEGYNALQYDATDDCVTCVEKNCPV	502
Hs-VSP1	WNLPPRRILFIKRFIIYSIR---GDVCDLVQVVMKKVVS--STSLGNCSILHDIETDKILINVDGPP	453
Hs-VSP1 _{CiV}	WNLPPRRILFIKRFIIYSIR---GDVCDLVQVVMKKVVS--STSLGNCSILHDIETDKVILINVDGPP	496
PTEN _{CiV}	-DYRPVALLFHKMM-FETI-PMFSGGTCNPQFVVCQLKVKIYSSNSG-----PTRREDKFMFYFEPQPLP	487
PTEN	-DYRPVALLFHKMM-FETI-PMFSGGTCNPQFVVCQLKVKIYSSNSG-----PTRREDKFMFYFEPQPLP	248
Ci-VSP	LAGDIKVRFMSTSKSLPRGYDNCPPYFWFNTSLVEGDH-----V	541
Hs-VSP1	LYDDVKVQFFS--SNLPKYDNCPPFFWFNTSFIQNNR-----L	490
Hs-VSP1 _{CiV}	LYDDVKVQFFS--SNLPKYDNCPPFFWFNTSFIQNNR-----L	533
PTEN _{CiV}	VCGDIKVEFFHKQNKMLK--KDKMFHFWVNTFFIPGPEETSEKVENGLCDQEIDSICSIERADNDKEYLVL	557
PTEN	VCGDIKVEFFHKQNKMLK--KDKMFHFWVNTFFIPGPEETSEKVENGLCDQEIDSICSIERADNDKEYLVL	318
	CBR3 loop	
Ci-VSP	TLKREEIDNPHKKKTWKIYRDNFTVKLTFSDAEDI-----	576
Hs-VSP1	CLPRNELDNPHKQKAWKIYPPEFAVEILFGEK-----	522
Hs-VSP1 _{CiV}	CLPRNELDNPHKQKAWKIYPPEFAVEILFGEK-----	565
PTEN _{CiV}	TLTKNDLDKANKDKANRYFSPNFVKLYFTKTVEEPSNPEASSSTSVTPDVSNDNEPDHYRSDTTSDPENE	629
PTEN	TLTKNDLDKANKDKANRYFSPNFVKLYFTKTVEEPSNPEASSSTSVTPDVSNDNEPDHYRSDTTSDPENE	390
Ci-VSP	-----	576
Hs-VSP1	-----	522
Hs-VSP1 _{CiV}	-----	565
PTEN _{CiV}	PFDEDQHSQITKV	642
PTEN	PFDEDQHTQITKV	403

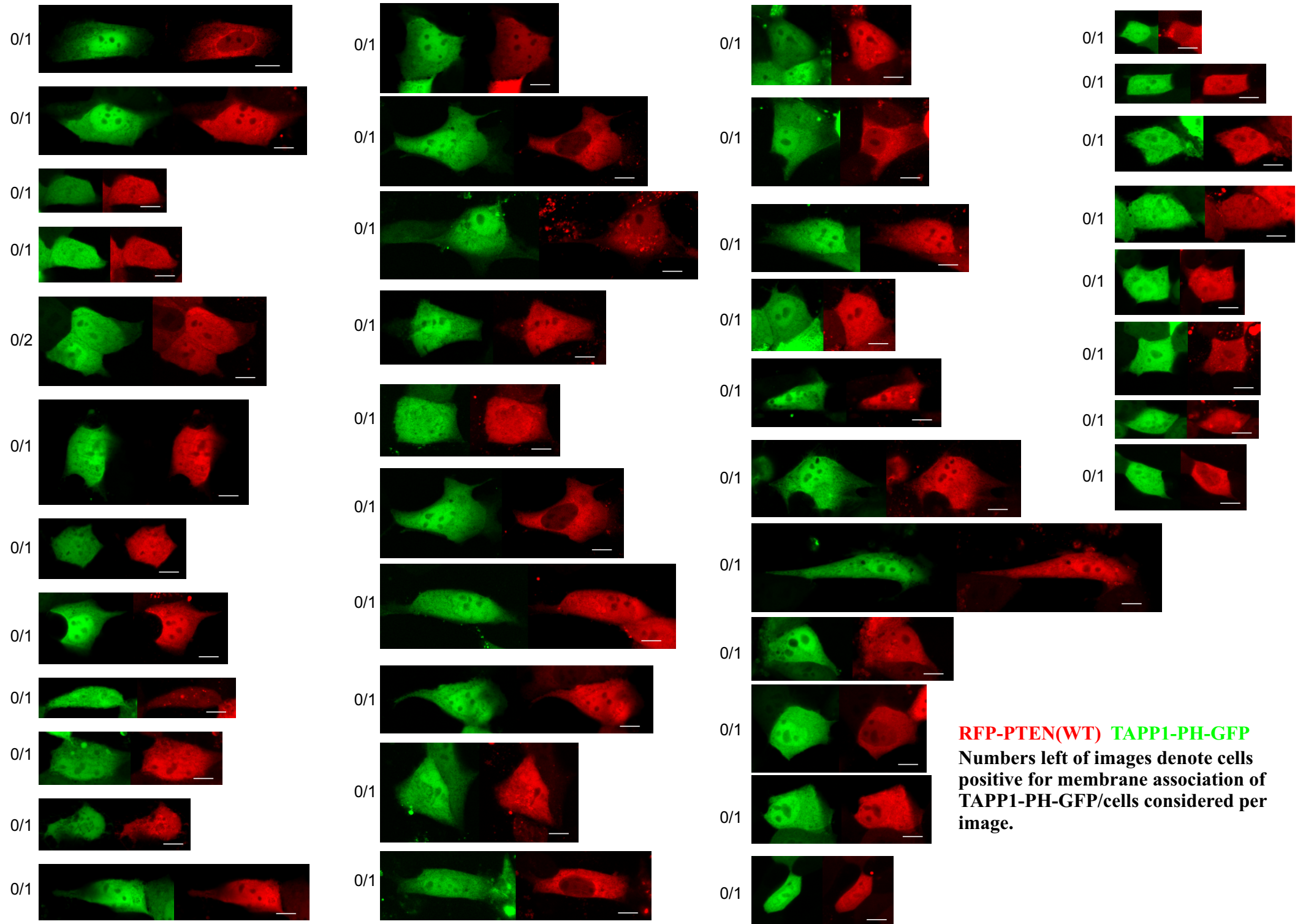
Supplemental Figure 1. Complete alignment of studied phosphatases and VSPs.

Supplemental Figure 1. Complete sequence alignment of studied phosphatases and VSPs.

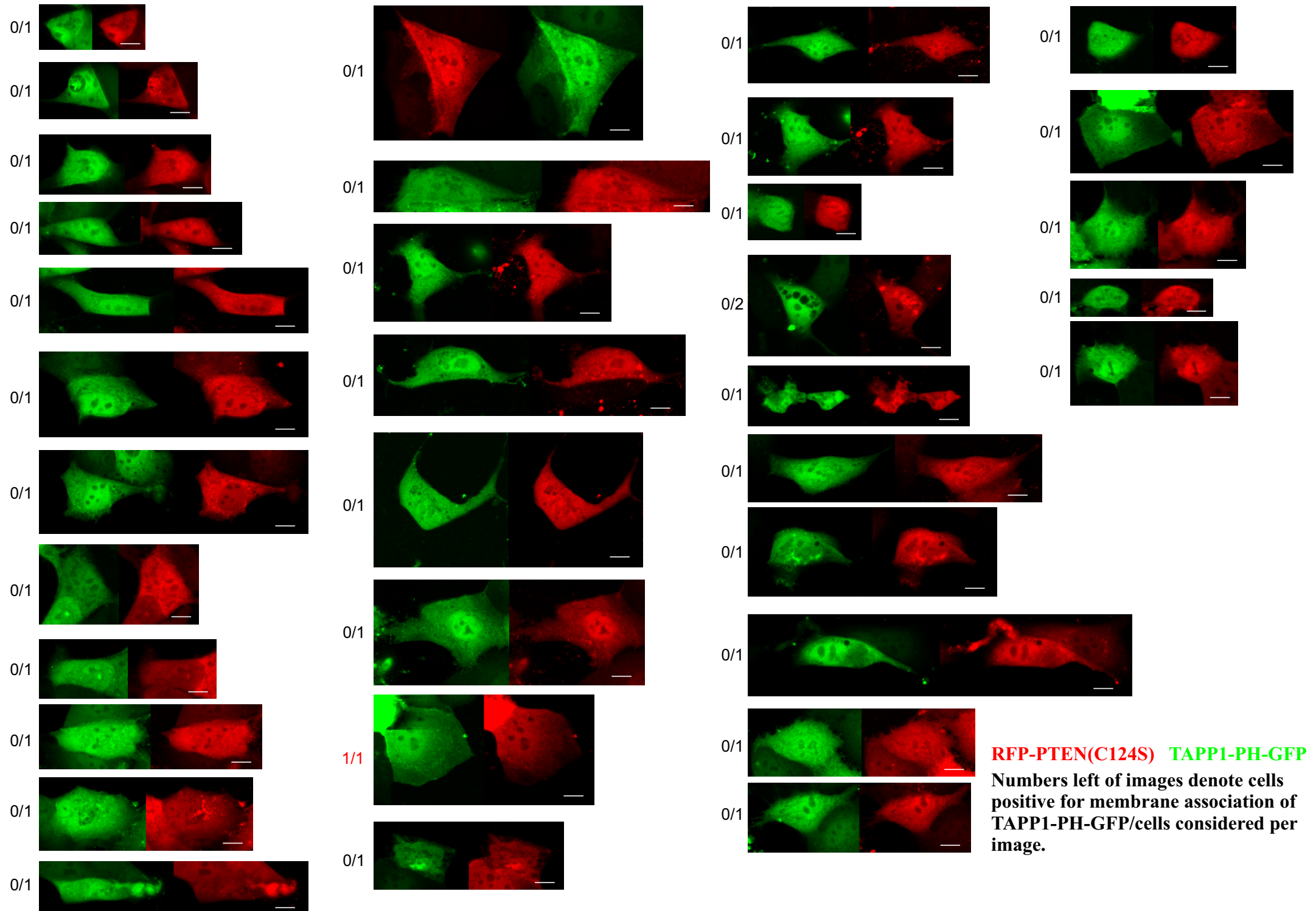
Sequence alignment of Ci-VSP, Hs-VSP1, Hs-VSP1_{CiV}, PTEN_{CiV} and PTEN as performed with ClustalX 2.1 [1] (UniProt accession numbers are: Q6XPS3, Hs-VSP1, also known as “TPTE2” or “TPIP”; P60484, human PTEN; Q4W8A1, Ci-VSP). N-terminus and voltage-sensor domain of Ci-VSP fused to the catalytic domain of Hs-VSP1 and PTEN are highlighted in *green* in the chimeras Hs-VSP1_{CiV} and PTEN_{CiV}. Please note that Hs-VSP1_{CiV}(D136N) mutant (termed Hs-VSP1_{CiV} or wild type) was used throughout this study (see also “Materials and Methods”). The D136N-mutation is highlighted in *magenta* in the alignment. Amino acids in loop regions forming substrate binding pocket (P, TI/ gating loop and WPD loop) are colored in *red*. Positions in P and TI/gating loop studied in this work are highlighted in *yellow*. Amino acids of CBR3-loop in the C2-domain are colored in *brown* with tyrosine (numbered Y522 in Ci-VSP) highlighted in *cyan*.

[1] Larkin MA, Blackshields G, Brown NP, et al (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948 . doi: 10.1093/bioinformatics/btm404

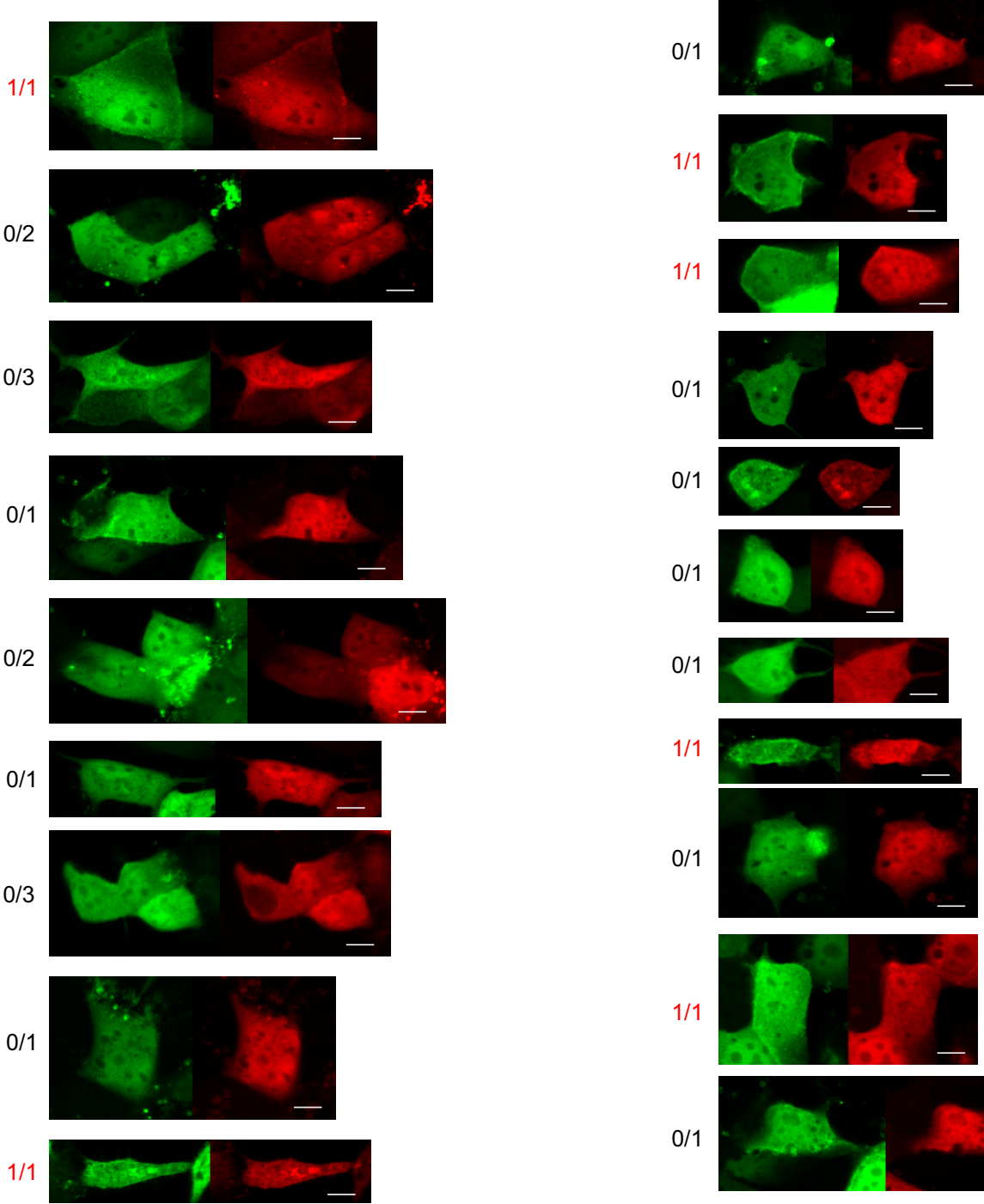
Supplemental Figure 2A. MDCK cells co-expressing RFP-PTEN(WT) together with TAPP1-PH-GFP.



Supplemental Figure 2B. MDCK cells co-expressing RFP-PTEN(C124S) together with TAPP1-PH-GFP.



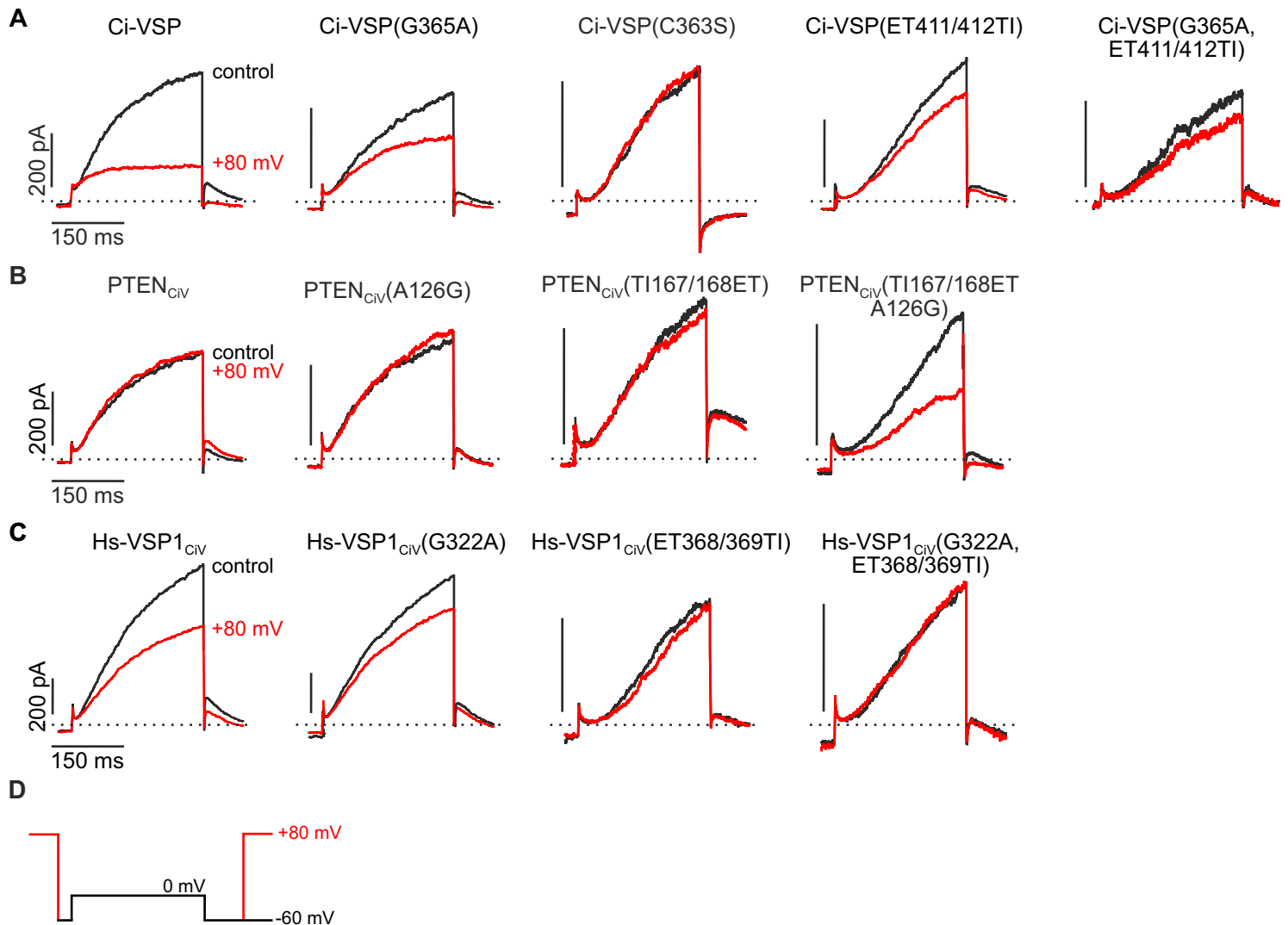
Supplemental Figure 2C. MDCK cells co-expressing RFP-PTEN(A126G, TI167/168ET) together with TAPP1-PH-GFP (page 2)



Supplemental Figure 2. Subcellular localization of TAPP1-PH-GFP in MDCK cells co-expressing soluble RFP-PTEN (wild type and mutants).

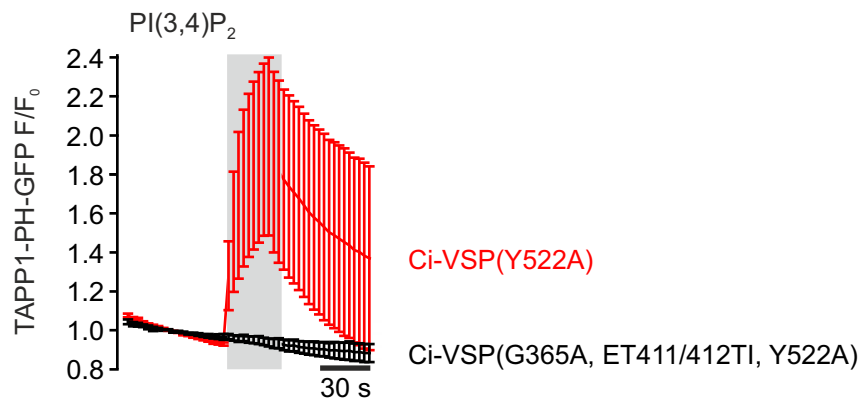
The PI(3,4)P₂-reporter TAPP1-PH-GFP was co-expressed in MDCK cells with N-terminally RFP-tagged (A) wild type PTEN, (B) PTEN(C124S) and (C) PTEN(A126G, TI167/168ET). Subcellular localization of the reporter was analyzed by confocal microscopy and image processing in ImageJ. This figure shows all cells analyzed in these experiments (all scale bars represent 10 μm). Quantification was done by blinded counting of cells with evident membrane association of TAPP1-PH-GFP (numbers left of single images indicate number of cells counted as positive for membrane association of TAPP1-PH-GFP from all cells considered per image). Summary of results: PTEN(wild type): 0 of 42 cells in total (0.0%); PTEN(C124S): 1 of 36 cells in total (2.8%); PTEN(A126G, TI167/168ET): 21 of 57 cells in total (36.8%).

RFP-PTEN(A126G, TI167/168E)-RFP TAPP1-PH-GFP
Numbers left of images denote cells positive for membrane association of TAPP1-PH-GFP/cells considered per image.



Supplemental Figure 3. Representative recordings of KCNQ2 (K_v7.2) currents in cells coexpressing VSPs. (A) KCNQ2 co-expressed with Ci-VSP wild type and mutants as indicated. Presented are KCNQ2 currents before (*black*) and at the end of Ci-VSP activation (*red*) (30 s at +80 mV). (B) Whole-cell currents in cells expressing KCNQ2 channels together with PTEN_{CIV} wild type and mutants (*black* and *red* trace represent control amplitudes and at the end of PTEN_{CIV} activation, respectively). (C) KCNQ2 currents before (*black*) and after (*red*) voltage-dependent activation of Hs-VSP1_{CIV} wild type and mutants. (D) Voltage protocol as employed for the recordings presented in (A-C). CHO cells were clamped at -60 mV and KCNQ2 currents were elicited every 5 s by a step depolarization to 0 mV. Under control conditions, the holding potential between the steps was constantly kept at -60 mV. To activate VSPs, the holding potential was changed to +80 mV in between the steps (indicated in *red*). Dashed line in panels A-C represents zero current.

Leitner et al., Figure S4



Supplemental Figure 4. Substrate specificity of Ci-VSP Y522 mutants.

Time course of the TIRF signal of TAPP1-PH-GFP co-expressed with Ci-VSP(Y522A) (*red trace*, $n=5$) or Ci-VSP(G365A, ET411/412TI, Y522A) (*black*, $n=5$). Phosphatases were activated by step depolarization to +80 mV for 30 s from a holding potential at -60 mV.