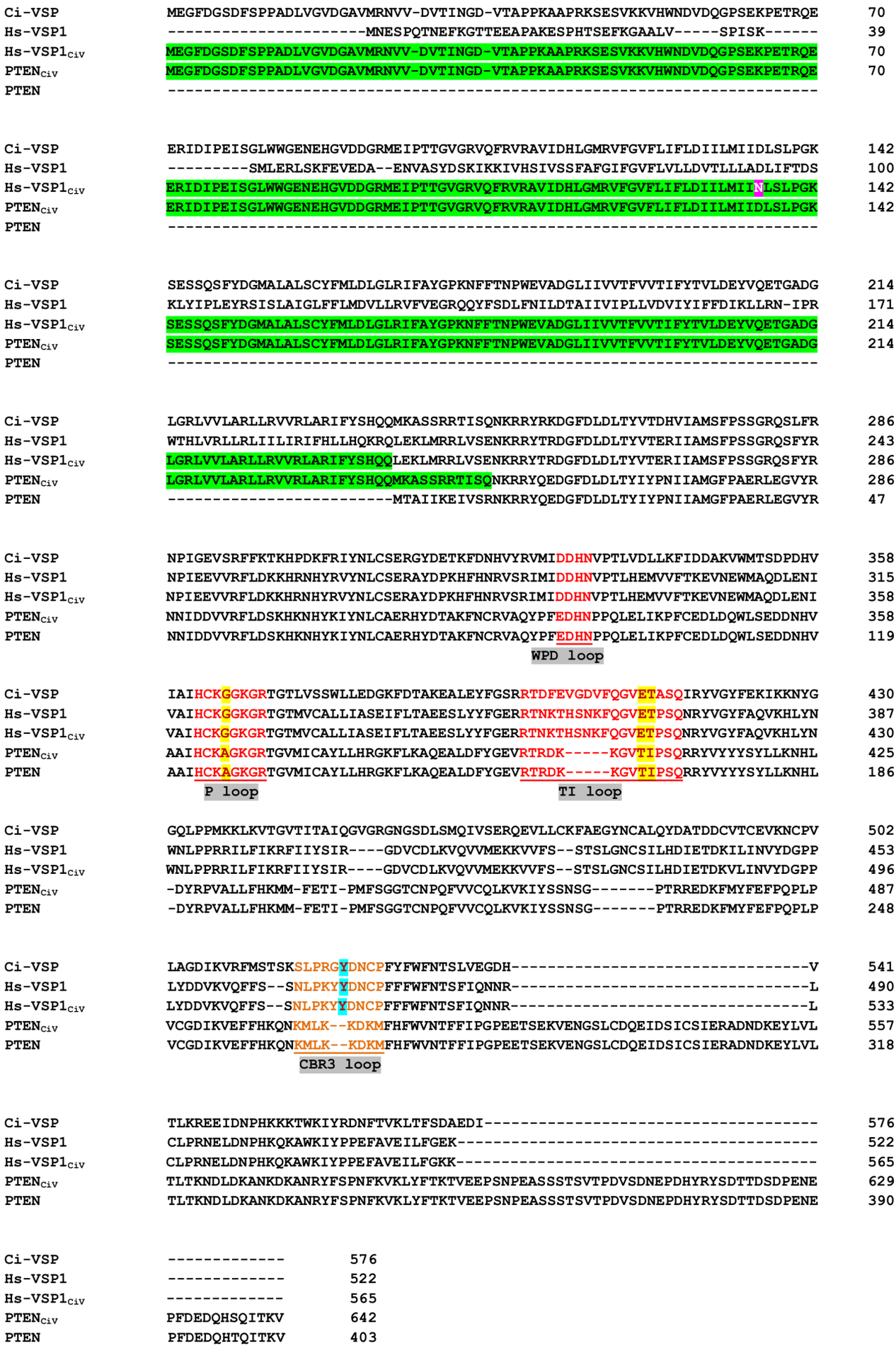


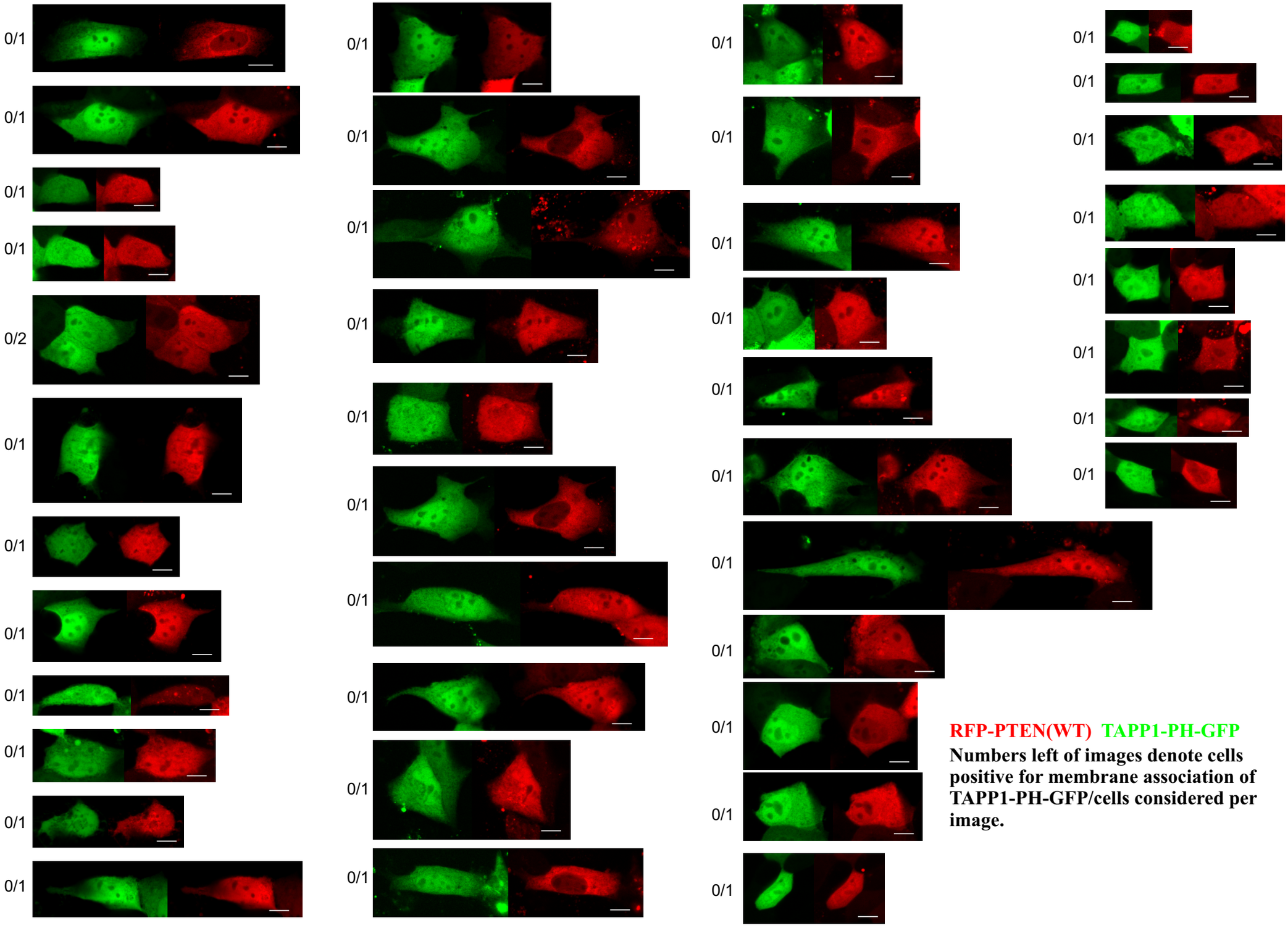
Supplemental Figure 1. Complete alignment of studied phosphatases and VSPs.

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

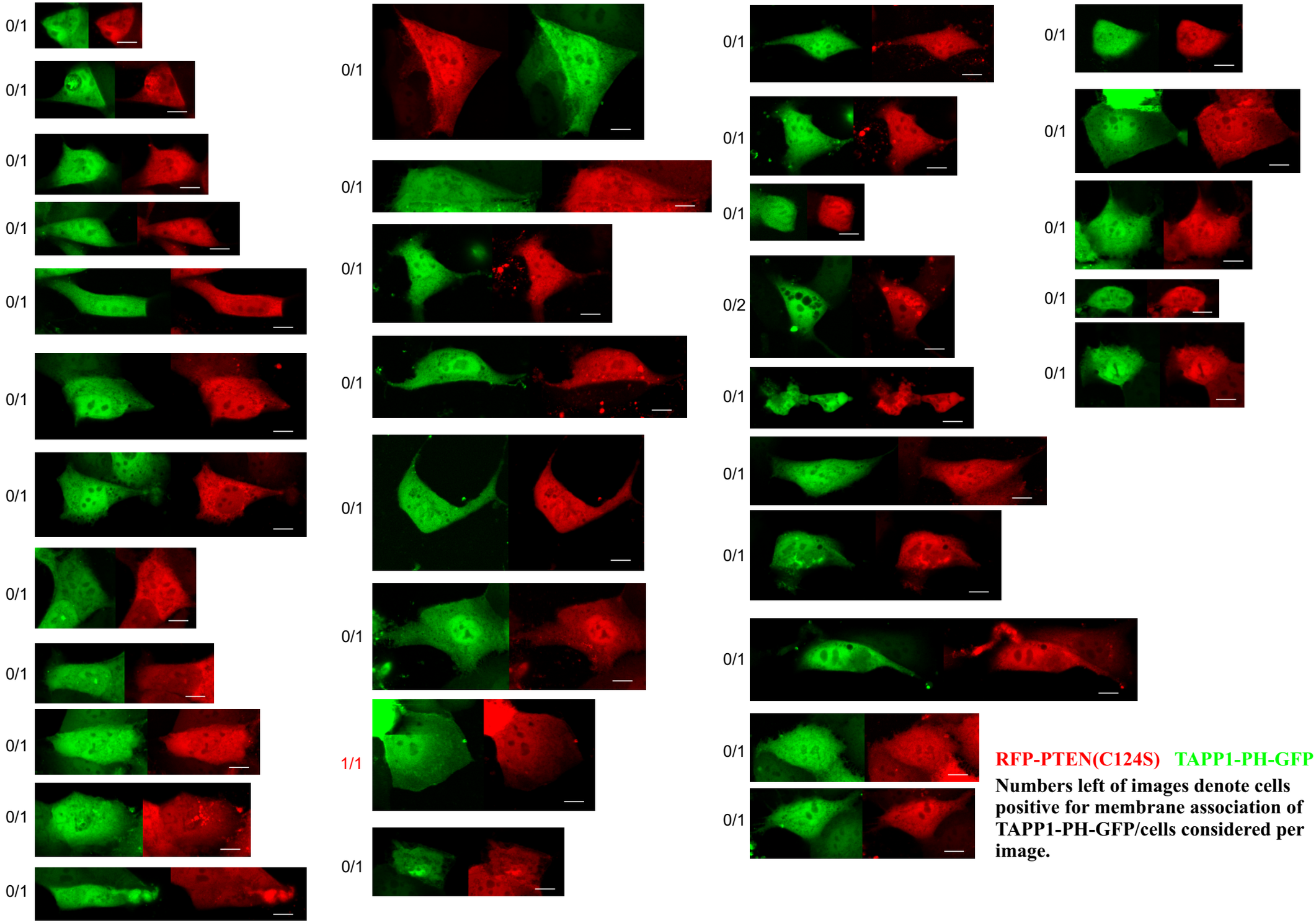
Sequence alignment of Ci-VSP, Hs-VSP1, Hs-VSP1$_{CiV}$, PTEN$_{CiV}$ and PTEN as performed with ClustalX 2.1 [1] (UniProt accession numbers are: Q6XPS3, Hs-VSP1, also known as "TPTE2" or "TPIP"; P60484, human PTEN; Q4W8A1, Ci-VSP). N-terminus and voltage-sensor domain of Ci-VSP fused to the catalytic 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

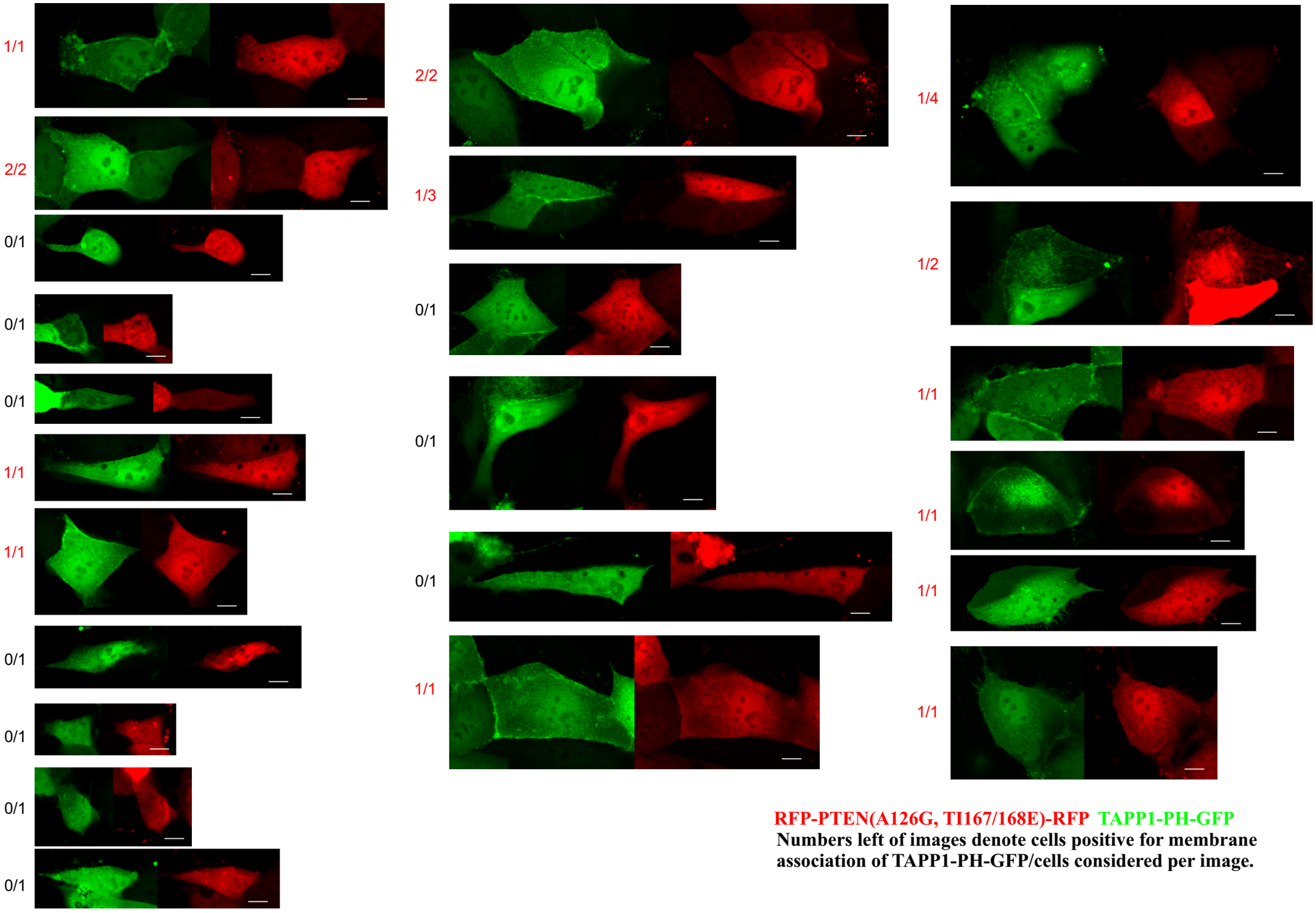
Supplemental Figure 2A. MDCK cells co-expressing RFP-PTEN(WT) together with TAPP1-PH-GFP.



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



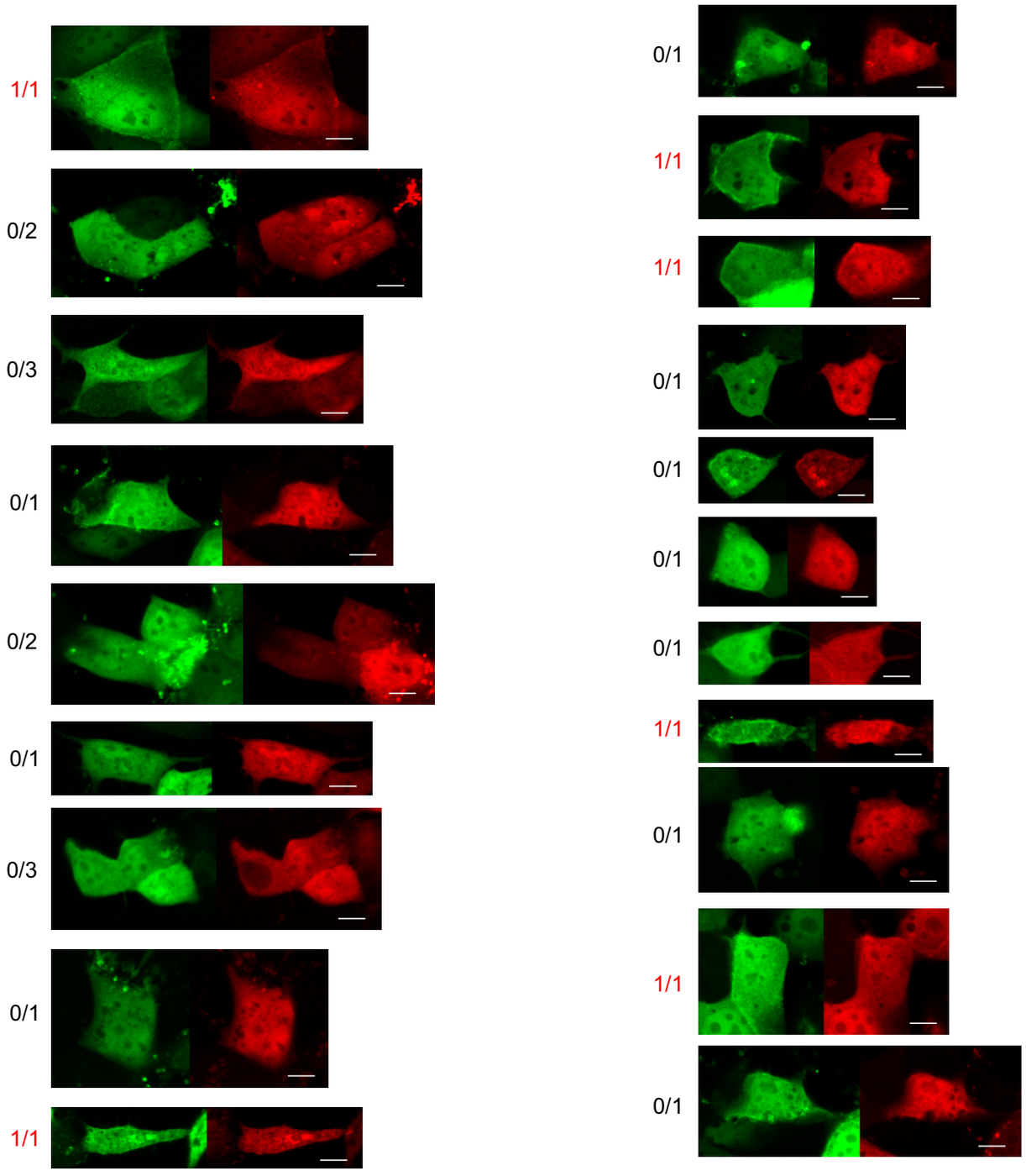
Supplemental Figure 2C. MDCK cells co-expressing RFP-PTEN(A126G, TI167/168ET) together with TAPP1-PH-GFP (page 1)



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.

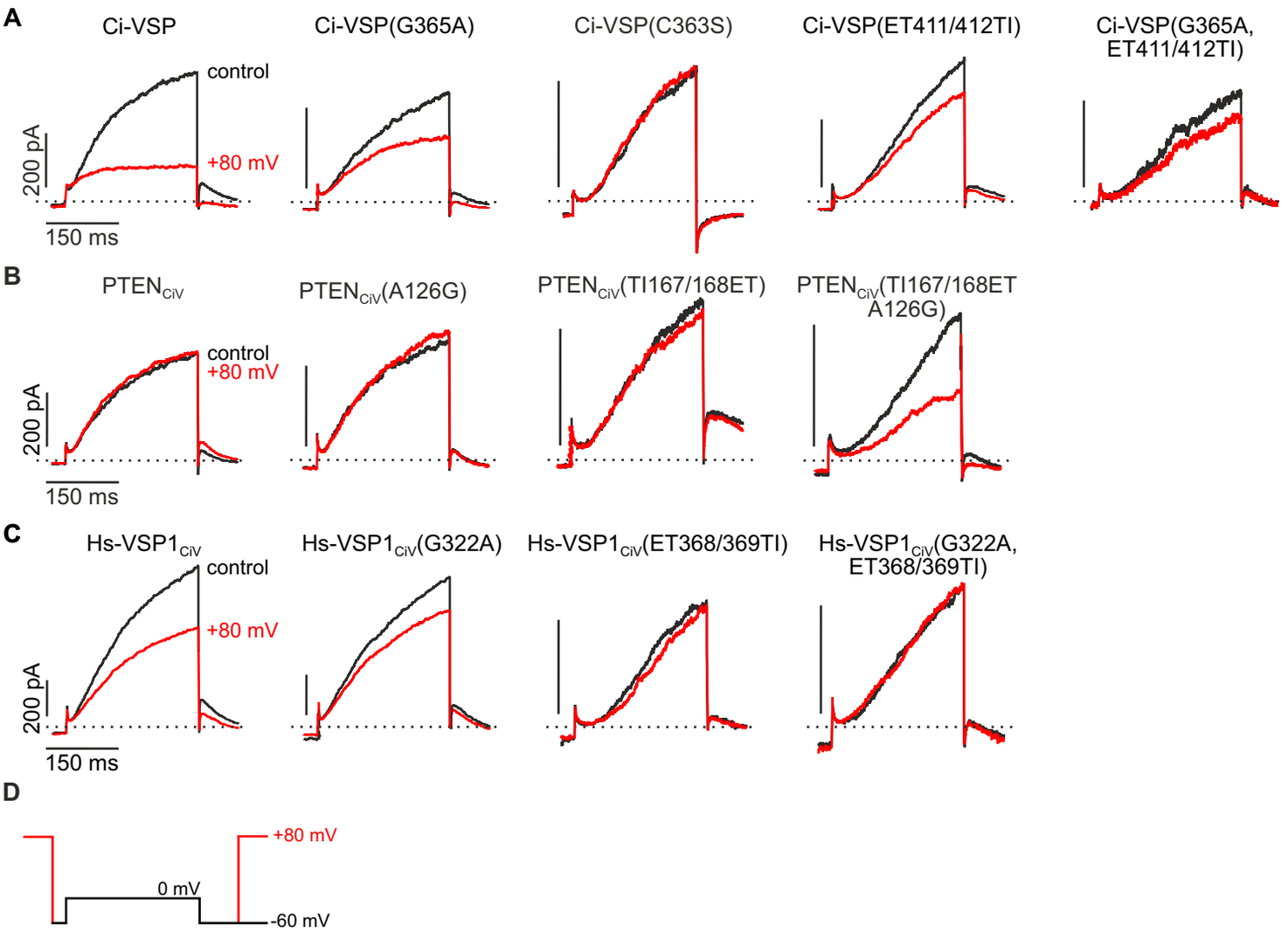
Supplemental Figure 2C. MDCK cells co-expressing RFP-PTEN(A126G, TI167/168ET) together with TAPP1-PH-GFP (page 2)



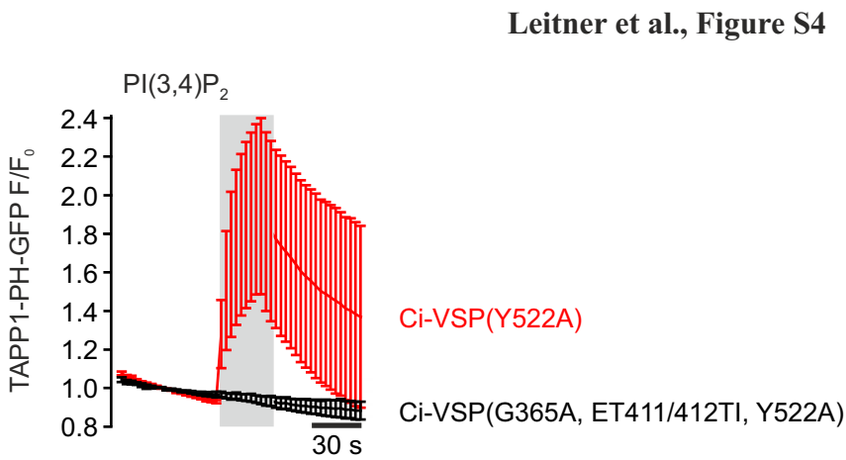
Supplemental Figure 2. Subcellular localization of TAPP1-PH-GFP in MDCK cells co-expressing soluble RFP-PTEN (wild type and mutants).

The PI(3,4)P_2-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.



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.