Supplemental Materials

Molecular Biology of the Cell

Pashkova *et al*.

Supplemental Figure 1. PRE effects on ^{15}N Ubiquitin.

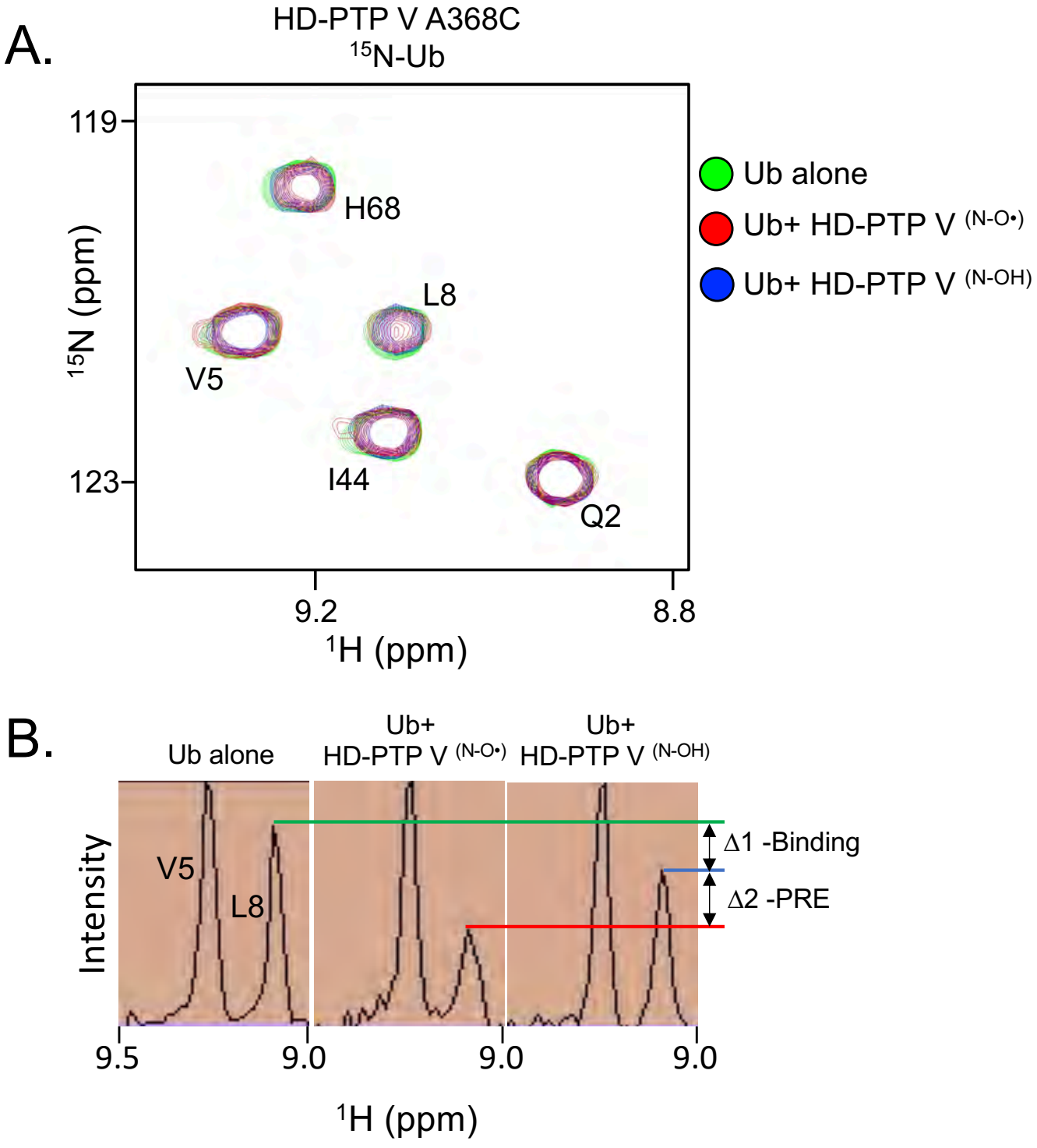
Top shows portion of HSQC of 30μM 15N Ub alone or in the presence 30μM VHDPTP spin-labelled at position 368 under oxidizing conditions or reduced in the presence of 2mM ascorbate. The backbone amide for L8 has a low peak height in the presence of spin-label that is partially restored in the presence of ascorbate. Binding of reduced V^{HDPTP} also reduces peak heights, which can also be observed in the slice view below. The extent of binding is reflected in decrease peak height in the presence of ascorbate (Δ1) and the PRE effect is reflected in the difference in peak height in the presence of V^{HDPTP} under oxidized and reduced conditions (Δ2).

Supplemental Figure 2. DEEPN Yeast 2-Hybrid analysis of WT and ΔUb V^{HDPTP}.

A. Abundance of reads for each potential interacting 'prey' gene in rank order for diploids carrying the Y2H pan-mouse cDNA library grown under permissive (His+) non-Y2H-selective conditions that contain the WT V^{HDPTP} as prey (x axis) vs the corresponding abundance of reads for those same genes for the population carrying the ΔUb V^{HDPTP} (Y-axis). These data demonstrate the variance in distribution of library plasmids betweeni the two populations that carry different V^{HDPTP} baits with an overall dispersion of 0.2.
B. Summary of in-frame open-reading frame fragments that enriched with high confidence (blue) when selected for interaction with WT or ΔUb V^{HDPTP}.
C. Data on each Y2H interactor in B. Shown is a plot of the nucleotide positions for each indicated cDNA sequence with the start and stop codon indicated. The left Y axis shows the read-depth for a 20 bp window along the cDNA sequence. The right Y axis shows the position and number of reads that bridge the 3' end of the Gal4-DNA binding domain and the 5' end of the interacting gene in blue. All fusion points were determined to be in-frame with the Gal4-DNA binding domain. Paired summary sequence data is shown for WT and ΔUb V^{HDPTP} on the right and left, respectively.

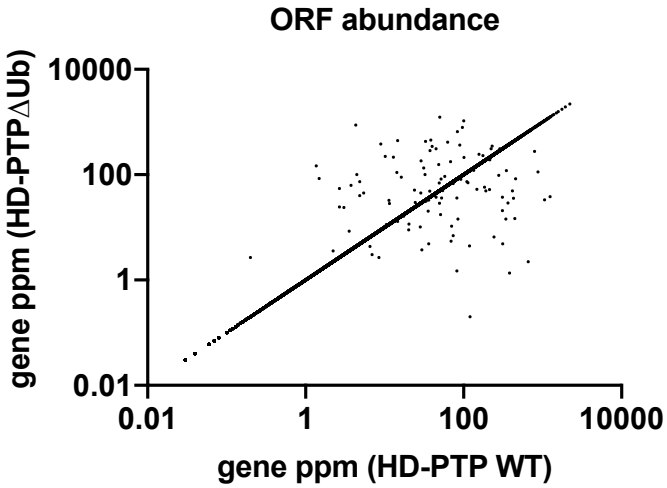
Supplemental Figure 3. HSCQ of ^{15}N-labelled Vps4 MIT domain bound to V domains.
HSQC spectra of 35μM ^{15}N-MIT domain from yeast Vps4 in the absence (green) and presence (red) of V^{Bro1}, V^{HDPTP}, and V^{Rim20} at the indicated ratios under the conditions described in Figure 8A

Supplemental Figure 1. *Pashkova et al. 2021*

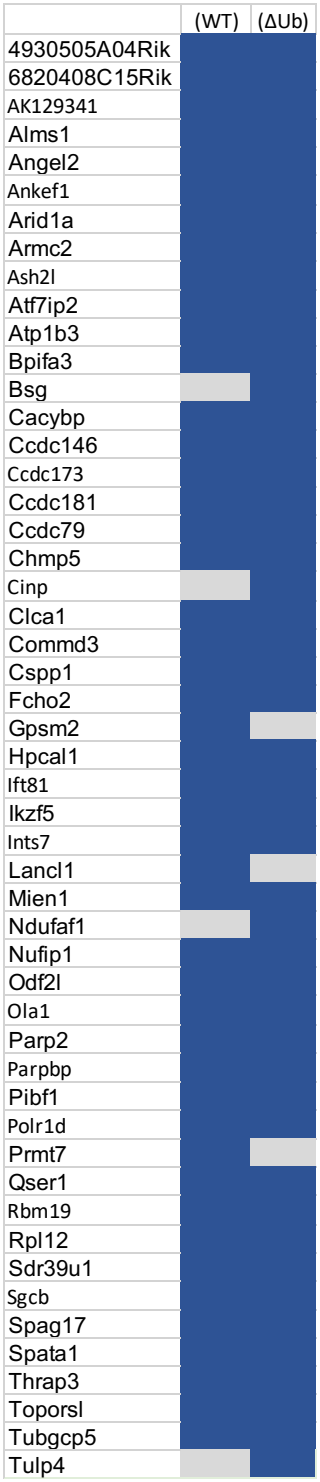


Supplemental Figure 2. *Pashkova et al. 2021*

A.

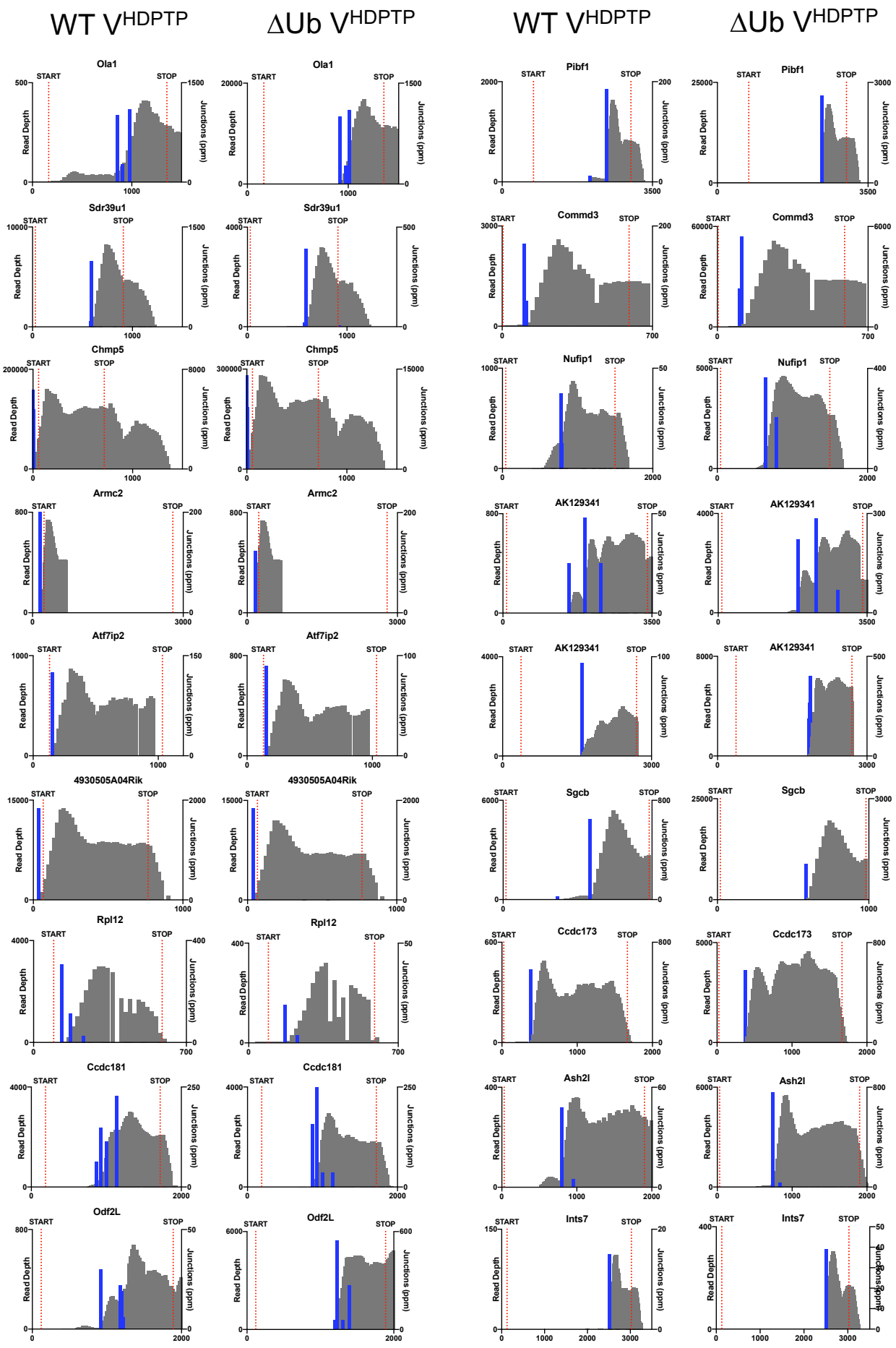


B.

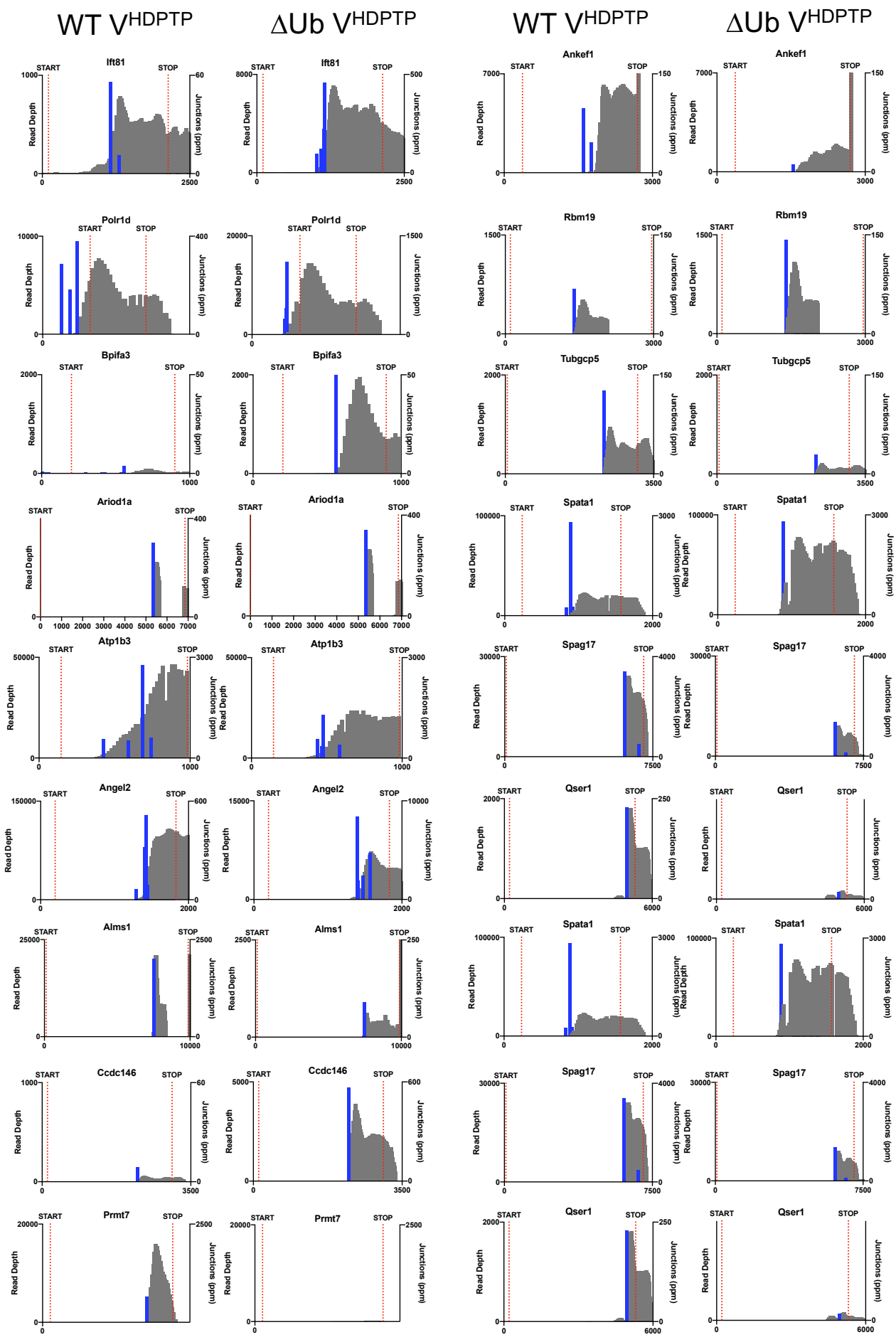


Supplemental Figure 2. *Pashkova et al. 2021*

C-1



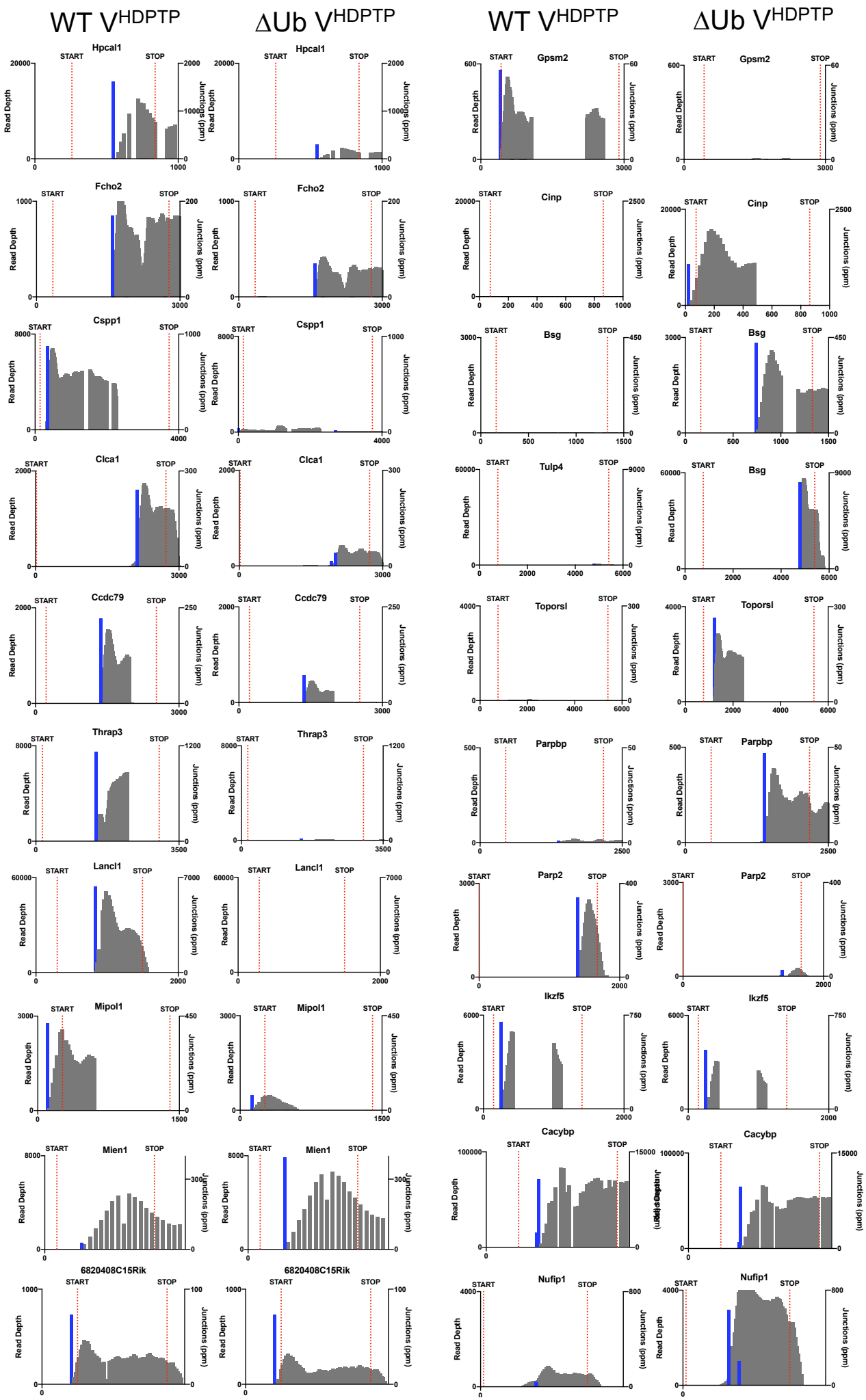
C-2



Supplemental Figure 2. *Pashkova et al. 2021*

C-3

Supplemental Figure 2. *Pashkova et al. 2021*



Supplemental Figure 3. *Pashkova et al. 2021*