

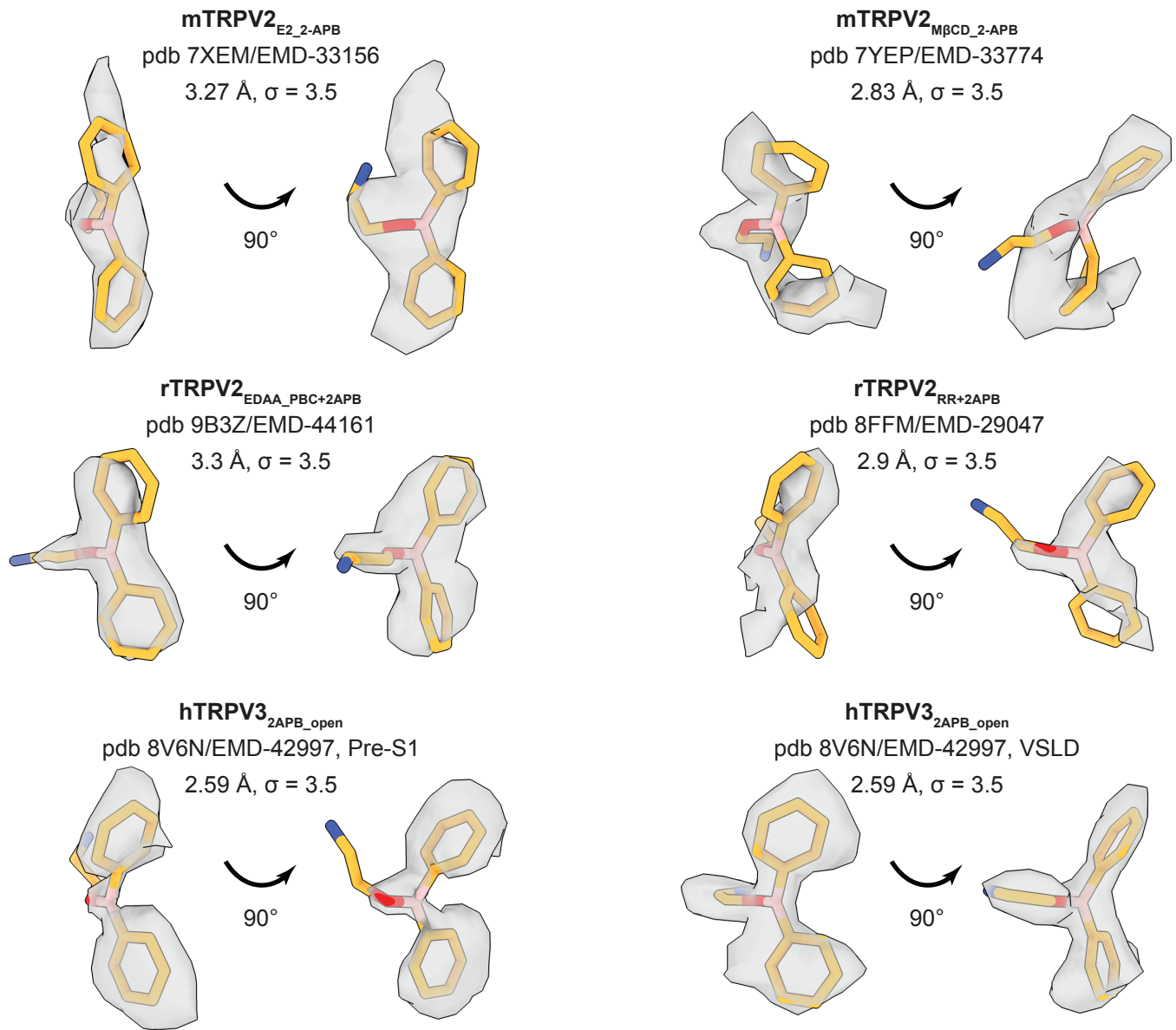
**Molecular determinants of 2-APB-sensitivity of TRPV2 - unexpected differences  
between two rodent orthologues**

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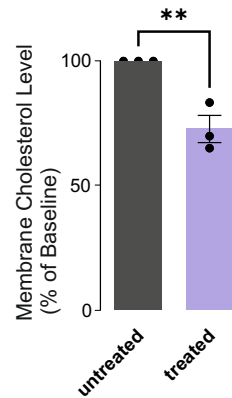


**Supplemental Figure 1: Comparison of 2-APB cryoEM density.** 2-APB density segmented from full cryoEM maps. Molecules and maps aligned to the central boron atom and the two connected carbons.

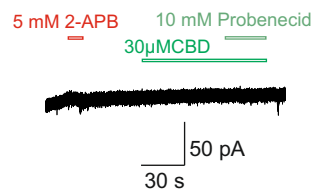
Rat	TRPV2	460	R-LFIWISFMDSY <b>Y</b> FEILFLQALLTVLSQVLRFMETEWYPLLVLSLVL <b>G</b> WLNLLYYTRGF <b>Q</b> H <b>T</b> GIYSVMI <b>Q</b> KVILRDLL	538
Mouse	TRPV2	455	R-LFIWISFMDSY <b>Y</b> FEILFLVQALLTVLSQVLRVETEWYPLLVSSVL <b>G</b> WLNLLYYTRGF <b>Q</b> H <b>T</b> GIYSVMI <b>Q</b> KVILRDLL	533
Human	TRPV2	458	H-VFIWISFIDSY <b>Y</b> FEILFLQALLTVVLSQVLCFLAIEWYPLLVSA <b>L</b> V <b>L</b> WLNLLYYTRGF <b>Q</b> H <b>T</b> GIYSVMI <b>Q</b> KVILRDLL	536
Human	TRPV1	500	R-PSMKT <b>L</b> FDVDSYSEMLFFLQSLFMLATVVLYF <b>S</b> HLKEYVASMV <b>F</b> SLAL <b>G</b> WLNMLYYTRGF <b>Q</b> MG <b>I</b> YAVMI <b>E</b> KMILRDLC	578
Human	TRPV3	510	P-SDLQ <b>S</b> ILSDAW <b>F</b> H <b>F</b> VFFIQAVLVILSVFLYLFAYKEYLAC <b>L</b> V <b>L</b> AMAL <b>G</b> WANMLYYTRGF <b>Q</b> SMG <b>M</b> YSVMI <b>Q</b> KVILHDVL	588
Human	TRPV4	536	KcPGVNS <b>L</b> FIDG <b>S</b> F <b>Q</b> LLYFIYSVLVIVSAALYLAGIEAYLAVMV <b>F</b> ALV <b>L</b> WLNALY <b>F</b> TRGL <b>K</b> L <b>T</b> GTYSIM <b>I</b> Q <b>K</b> ILFKDLF	615
Rat	TRPV2	539	<b>R</b> FLLVYLVFLFGFAVALVLSREARSPKAPED--NNSTVTEQPTVGQEEEP--APYRSILDASLELFKFTIGMGELAFQE	614
Mouse	TRPV2	534	<b>R</b> FLLVYLVFLFGFAVALVLSREARSPKAPED--SNTTVTEKPTLGQEEEP--VPYGGILDASLELFKFTIGMGELAFQE	609
Human	TRPV2	537	<b>R</b> FLLIYLVFLFGFAVALVLSQEAWRPEAPT <b>G</b> --PNATESVQPMEGQ <b>E</b> DEGN <b>g</b> AQYRGILEASLELFKFTIGMGELAFQE	614
Human	TRPV1	579	<b>R</b> FMFVYIVFLFGFSTAVVTLIEDGKND <b>S</b> LPSE--STSHRWRGPACRPPD <b>S</b> ---YNSLYSTCLELFKFTIGMGDLEFTE	652
Human	TRPV3	589	<b>K</b> FLFVYIVFLLGF <b>G</b> VALASLIE-----KCPKD-----N <b>K</b> DCSS---YGSFSDAVLELFKLTIGLGD <b>L</b> NIQQ	646
Human	TRPV4	616	<b>R</b> FLLVYLLFMIGYASALVSLNPCANMKVCNE <b>d</b> qTNCTVPTYPSCR <b>D</b> SET <b>F</b> S-----TFLLDLFKLTIGMGDLE <b>M</b> LS	687
Rat	TRPV2	615	QLRFRGVVLLLLL <b>L</b> AYV <b>I</b> LYVLLLNMLIALMSETVNVHADNSWSIWKLQK <b>A</b> ISVLEMENGYW <b>W</b> CRRKKHREGRL <b>L</b> KVGTR	694
Mouse	TRPV2	610	QLRFRGVVLLLLL <b>L</b> AYV <b>I</b> LYVLLLNMLIALMSETVNSVATDSWSIWKLQK <b>A</b> ISVLEMENGYW <b>W</b> CRRKRHRAGRL <b>L</b> KVGTK	689
Human	TRPV2	615	QLHFRGMVLLLLL <b>L</b> AYV <b>I</b> LYI <b>L</b> LNMLIALMSETVNSVATDSWSIWKLQK <b>A</b> ISVLEMENGYW <b>W</b> CR <b>K</b> KQ--RAGV <b>M</b> LT <b>V</b> GTK	693
Human	TRPV1	653	NYDFKAVFI <b>L</b> LLAYV <b>I</b> LYI <b>L</b> LNMLIALMGETVN <b>K</b> IAQESKNIWKLQ <b>R</b> AITILDTEKSFLK <b>C</b> MRKAFRSGK <b>L</b> LQVGYT	732
Human	TRPV3	647	NSKYPI <b>L</b> FLFL <b>L</b> ITYV <b>I</b> LYVLLLNMLIALMGETVEN <b>V</b> SKESER <b>I</b> WRLQ <b>R</b> ARTILEFEK <b>M</b> LPEW <b>L</b> RSRFRMGEL <b>C</b> KVA--	724
Human	TRPV4	688	STKYPV <b>V</b> FI <b>L</b> L <b>V</b> TY <b>I</b> LYVLLLNMLIALMGETV <b>G</b> QVSKES <b>K</b> HIWKLQ <b>W</b> ATTILD <b>I</b> ERS <b>F</b> FPV <b>L</b> RKA <b>F</b> RS <b>G</b> EM <b>V</b> T <b>V</b> GTKS	767

**Supplemental Figure 2: Multiple sequence alignment of TRPV channels.** Multiple sequence alignment of TRPV channels highlighting 2-APB interaction sites. Amino acid sequences of rat (rTRPV2), mouse (mTRPV2) and human (hTRPV2) were aligned alongside human TRPV1, TRPV3, and TRPV4 to compare similar and divergent regions relevant to ligand interaction. Residues previously implicated in 2-APB binding within the VBP are highlighted in red, and the residues implicated for RTX-sensitivity in green. The residues from the S5 binding domain involved in 2-APB interaction are highlighted in blue. This alignment illustrates the degree of sequence similarity across TRPV family members and highlights the mutation sites investigated in this study.

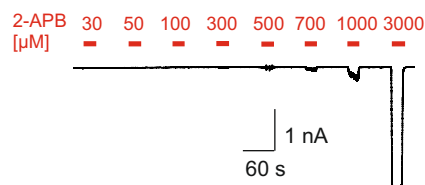
### A Cholesterol Depletion



### B HEK 293t

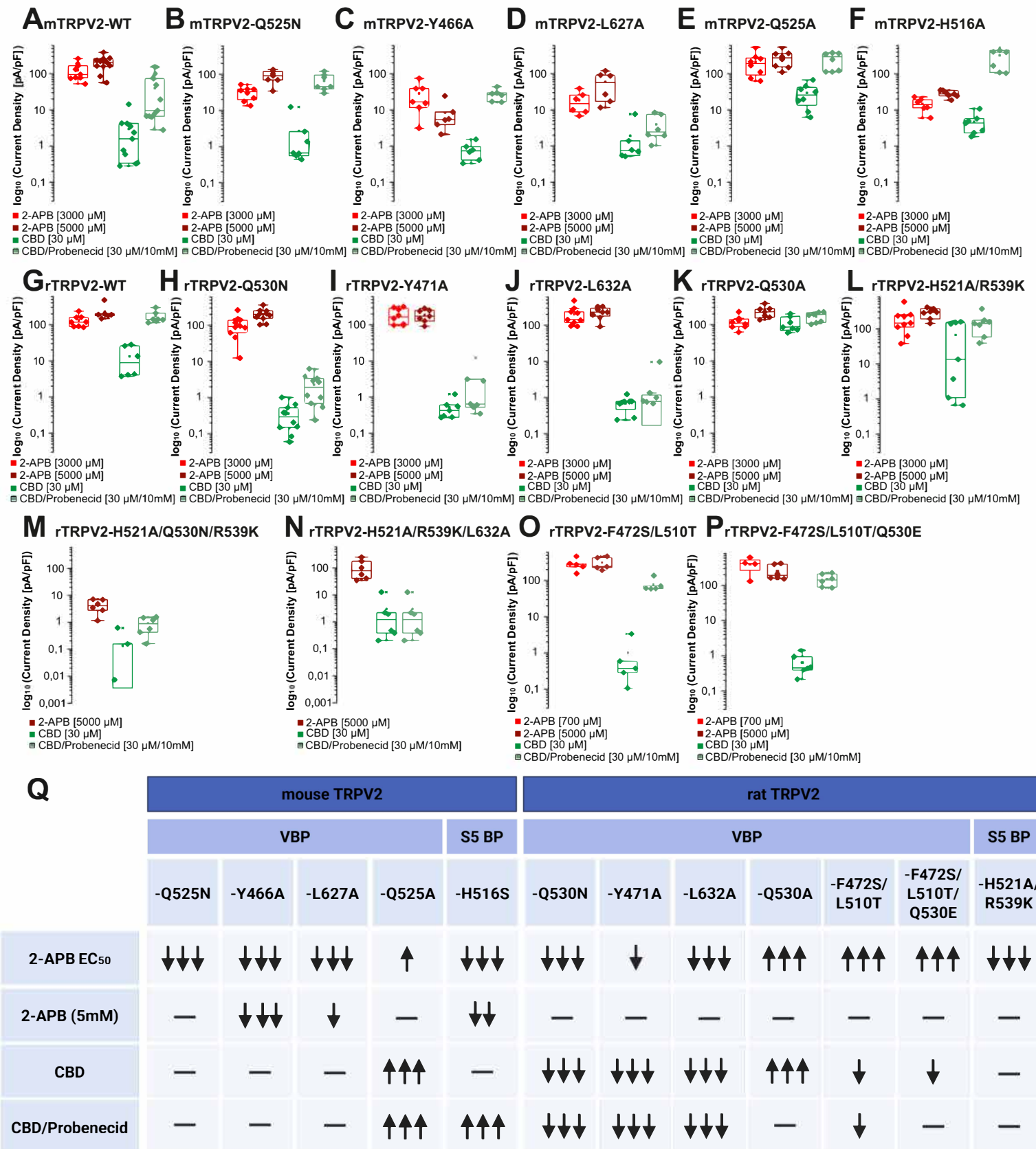


### C rTRPV2-H521A/R539K



#### Supplemental Figure 3:

**A.** Effect of methyl- $\beta$ -cyclodextrin treatment on membrane cholesterol levels in HEK 293 cells. HEK 293 cells were either left untreated (baseline control, set to 100%) or treated with 10 mM methyl- $\beta$ -cyclodextrin (M $\beta$ CD) for 15 minutes to deplete membrane cholesterol. Bars represent mean  $\pm$  SEM of membrane cholesterol levels expressed as a percentage of the untreated control. The treated group (shown in lila) exhibited a ~27% reduction in membrane cholesterol compared to baseline. **B.** Representative whole-cell patch-clamp current trace displaying an application of 5 mM 2-APB followed by 30  $\mu$ M CBD and the co-application of 10 mM probenecid in native HEK 293t cells. **C.** Sample current trace from whole-cell patch-clamp experiments showing concentration-dependent activation of the rTRPV2 mutant H521A-R539K by 2-APB. \* $p$  < 0.05; \*\* $p$  < 0.01; \*\*\* $p$  < 0.001; and n.s. not significant.



**Supplemental Figure 4:** Comparison of current densities evoked by 700  $\mu\text{M}$  or 3000  $\mu\text{M}$  2-APB (red), 5000  $\mu\text{M}$  2-APB (brown), 30  $\mu\text{M}$  CBD (dark green), and co-application of CBD (30  $\mu\text{M}$ ) with Probenecid (10 mM) (light green) for mouse and rat TRPV2 wild-type and mutant channels. Current densities are plotted on a log-scale to allow for direct comparison across stimuli. Data are shown for mTRPV2 - WT (**A**), - Q525N (**B**), - Y466A (**C**), - L627A (**D**), - Q525A (**E**), - H516A (**F**); rTRPV2 - WT (**G**), - Q530N (**H**), - Y471A (**I**), - L632A (**J**), - Q530A (**K**), - H521A/R539K (**L**), - H521A/Q530N/R539K (**M**), - H521A/R539K/L632A (**N**), F472S/L510T (**O**), - F472S/L510T/Q530E (**P**). **Q**. The table compares the response of mouse (- Q525N, - Y466A, - L627A, - Q525A, - H516S) and rat (- Q530N, - Y471A, - L632A, - Q530A, - H521A/R539K, - F472S/L510T, - F472S/L510T/Q530E) TRPV2 mutants to wild-type channels under stimulation with 2-APB (estimated EC<sub>50</sub> and 5mM), CBD, or the co-application of CBD and probenecid. Arrows indicate the direction and significance of the effect relative to the wild-type of the corresponding species: — = no significant difference; ↓ =  $p < 0.05$ ; ↑↑↑ or ↓↓↓ =  $p < 0.001$ . Created in BioRender. Leffler, A. (2025) [https:// BioRender.com/9pye8mq](https://BioRender.com/9pye8mq).