**Table S6: Summary of alternative splice variants impacting regions of the catalytic dyad.**

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| **Catalytic Dyad (GXSX + H)** | |  |  |
| **Rhomboid Name (accession #'s) [total forms]** | **Species** | **Rhomboid Type** | **Effect of Splicing** |
| Isoform 3 (NM\_001324436.1/NP\_001311365.1) [10 forms] | Humans | PARL | Removal of 15 residues from the TMD catalytic GASG leaving only GA, plus a frameshift in TMD7 that extended TMD7 by 8 residues |
| Isoform 4 (NM\_001324437.1/NP\_001311366.1) [10forms] | Humans | PARL | Removal of 15 residues from the TMD catalytic GASG leaving only GA, plus a frameshift in TMD7 that extended TMD7 by 8 residues |
| Isoform 5 (NM\_001324438.1/NP\_001311367.1) [10 forms] | Humans | PARL | Catalytic site changed from GASG to GAMV with an early termination of the protein sequence |
| X1 PARL (XM\_017006800.1/XP\_016862289.1) [10 forms] | Humans | PARL | Removal of 15 residues from the TMD catalytic GASG leaving only GA, plus a frameshift in TMD7 that extended TMD7 by 8 residues |
| X2 PARL (XM\_005247582.4/XP\_005247639.1) [10 forms] | Humans | PARL | A frameshift in TMD7 extends that extended TMD7 by 8 residues |
| X3 PARL (XM\_017006802.1/XP\_016862291.1) [10 forms] | Humans | PARL | Removal of 15 residues from the TMD catalytic GASG leaving only GA, plus a frameshift in TMD7 that extended TMD7 by 8 residues |
| X4 PARL (XM\_017006801.1/XP\_016862290.1) [10 forms] | Humans | PARL | A frameshift in TMD7 extends that extended TMD7 by 8 residues |
| X5 PARL (XM\_017006803.1/XP\_016862292.1) [10 forms] | Humans | PARL | A frameshift in TMD7 extends that extended TMD7 by 8 residues |
|  |  |  |  |
| X6 RHBDF1 iRhom1 (XM\_017023558.1/XP\_016879047.1) [7 forms] | Human | iRhom (evolved from PARL) | No catalytic dyad |
|  |  |  |  |
| X1 RHBDL1 (XM\_017023849.1/XP\_016879338.1) [5 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a unique sequence |
| X2 RHBDL1 (XM\_017023850.1/XP\_016879339.1) [5 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in the removal of the dyad |
|  |  |  |  |
| X1 RHBDL3 (XM\_017024272.1/XP\_016879761.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X2 RHBDL3 (XM\_017024275.1/XP\_016879764.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X3 RHBDL3 (XM\_017024273.1/XP\_016879762.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X4 RHBDL3 (XM\_017024276.1/XP\_016879765.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X9 RHBDL3 (XM\_017024274.1/XP\_016879763.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X10 RHBDL3 (XM\_017024278.1/XP\_016879767.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X11 RHBDL3 (XM\_017024277.1/XP\_016879766.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
| X12 RHBDL3 (XM\_017024280.1/XP\_016879769.1) [13 forms] | Human | Secretase-type (6+1) | A frameshift that resulted in a different sequence |
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| Isoform b DERL1 (NM\_001134671.2/NP\_001128143.1) [3 forms] | Human | Rhomboid pseudoprotease | Whole TMD6 is missing |
|  |  |  |  |
| Isoform c DERL2 (NM\_001304779.1/NP\_001291708.1) [3 forms] | Human | Rhomboid pseudoprotease | Whole TMD6 is missing |
|  |  |  |  |
| X3 DERL3 (XM\_017029080.1/XP\_016884569.1) [10 forms] | Human | Rhomboid pseudoprotease | Additional residues, plus a unique sequence |
| X4 DERL3 (XM\_017029079.1/XP\_016884568.1) [10 forms] | Human | Rhomboid pseudoprotease | Additional residues, plus a unique sequence |
| X5 DERL3 (XM\_017029078.1/XP\_016884567.1) [10 forms] | Human | Rhomboid pseudoprotease | Additional residues, plus a unique sequence |
| X7 DERL3 (XM\_017029081.1/XP\_016884570.1) [10 forms] | Human | Rhomboid pseudoprotease | Different sequence |
|  |  |  |  |
| X1 Rhbf1 iRhom1 (XM\_006514492.1/XP\_006514555.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X2 Rhbf1 iRhom1 (XM\_006514493.1/XP\_006514556.1) [13 forms] | Mouse | iRhom (evolved from PARL) | A frameshift within TMD2 which potentially generated an unrelated peptide sequence |
| X3 Rhbf1 iRhom1 (XM\_006514494.1/XP\_006514557.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X4 Rhbf1 iRHom1 (XM\_006514495.1/XP\_006514558.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X5 Rhbf1 iRhom1 (XM\_006514496.1/XP\_006514559.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X7 Rhbf1 iRhom1 (XM\_006514498.1/XP\_006514561.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X8 Rhbf1 iRhom1 (XM\_006514499.1/XP\_006514562.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X10 Rhbf1 iRhom1 (XM\_006514501.1/XP\_006514564.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
| X12 Rhbf1 iRhom1 (XM\_006514503.1/XP\_006514566.1) [13 forms] | Mouse | iRhom (evolved from PARL) | Additional 2 residues within TMD4 (GPAG) and loss of 1 residue within TMD6 as well the H residue is converted to a P amino acid |
|  |  |  |  |
| X1 Rhbdf2 iRhom2 XM\_006533108.1/XP\_006533171.1) [3 forms] | Mouse | iRhom (evolved from PARL) | A frameshift within TMD2 resulted in sequence changes downstream |
|  |  |  |  |
| X1 Rhbdl3 (XM\_006533326.1/XP\_006533389.1) [7 forms] | Mouse | Secretase-type (6+1) | A frameshift within TMD3 changes catalytic dyad and the entire sequence |
| X2 Rhbdl3 (XM\_006533327.1/XP\_006533390.1) [7 forms] | Mouse | Secretase-type (6+1) | A frameshift within TMD3 changes catalytic dyad and the entire sequence |
| X3 Rhbdl3 (XM\_006533328.1/XP\_006533391.1) [7 forms] | Mouse | Secretase-type (6+1) | A frameshift within TMD3 changes catalytic dyad and the entire sequence |
| X6 Rhbdl3 (XM\_006533331.1/XP\_006533394.1) [7 forms] | Mouse | Secretase-type (6+1) | A frameshift within TMD3 changes catalytic dyad and the entire sequence |
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| At1g74130 (NM\_202413.1/NP\_974141.1) [4 forms] | Arabidopsis | Inactive homologue | A frameshift that resulted in the introduction of unique residues, plus an early termination that resulted in the removal of the catalytic Q residue |
| At1g74130 (S) [4 forms] | Arabidopsis | Inactive homologue | A frameshift that resulted in the introduction of unique residues, plus an early termination that resulted in the removal of the catalytic Q residue |
| At1g74130 (M) [4 forms] | Arabidopsis | Inactive homologue | A frameshift that resulted in the introduction of unique residues, plus an early termination that resulted in the removal of the catalytic Q residue |
|  |  |  |  |
| RBL14 At3g17611 (NM\_001084701.1/NP\_001078170.1) [3 forms] | Arabidopsis | Secretase (basic) | Alternate methionine used and the sequence starts after the catalytic GFSG instead |
|  |  |  |  |
| RBL3 At5g07250 (NM\_001125710.1/NP\_001119182.1) [2 forms] | Arabidopsis | Secretase-type (6+1) | TMD6 which contained a spliced out catalytic H, and a restoration of the sequence downstream |
|  |  |  |  |
| IsoformB ROM-4 (NM\_001047549.2 / NP\_001041014.1) [3 forms] | C elegans | Secretase (basic) | Alternate start methionine used that resulted in a frame shift within the L1 loop that created additional residues and then a restoration of the downstream sequence |