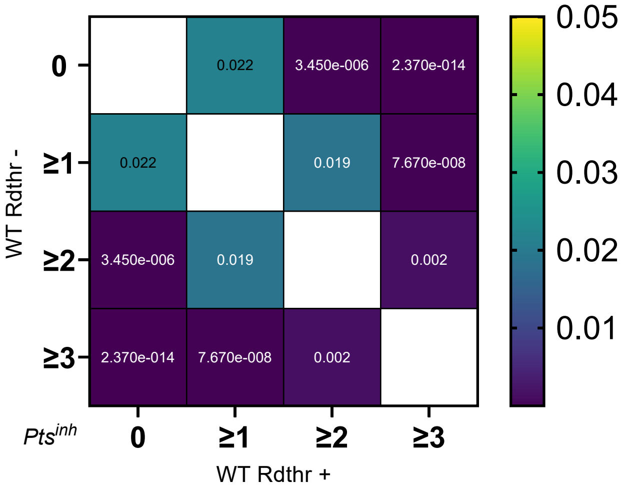
# Supplementary data

## Supplementary Table S2

## Distribution of Pts$^{inh}$ based on WT readthrough

**A**

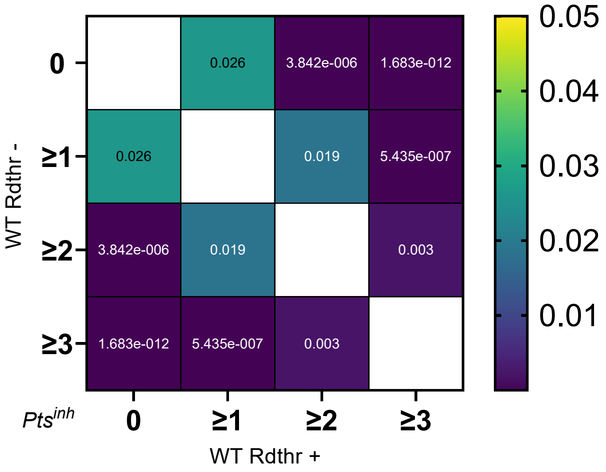
| *WT Rdthr* | *0* | *≥1* | *≥2* | *≥3* |
|---|---|---|---|---|
| + | 164 | 31 | 10 | 0 |
| *(%)* | *(20.1)* | *(13.4)* | *(6.0)* | *(0.0)* |
| - | 653 | 200 | 156 | 136 |
| *Total* | *817* | *231* | *166* | *136* |



| | 0 | ≥1 | ≥2 | ≥3 |
|---|---|---|---|---|
| 0 | | 0.022 | 3.450e-006 | 2.370e-014 |
| ≥1 | 0.022 | | 0.019 | 7.670e-008 |
| ≥2 | 3.450e-006 | 0.019 | | 0.002 |
| ≥3 | 2.370e-014 | 7.670e-008 | 0.002 | |

**B**

| *WT Rdthr* | *0* | *≥1* | *≥2* | *≥3* |
|---|---|---|---|---|
| + | 116 | 31 | 10 | 0 |
| *(%)* | *(20.4)* | *(13.4)* | *(6.0)* | *(0.0)* |
| - | 454 | 200 | 156 | 136 |
| *Total* | *570* | *231* | *166* | *136* |



| | 0 | ≥1 | ≥2 | ≥3 |
|---|---|---|---|---|
| 0 | | 0.026 | 3.842e-006 | 1.683e-012 |
| ≥1 | 0.026 | | 0.019 | 5.435e-007 |
| ≥2 | 3.842e-006 | 0.019 | | 0.003 |
| ≥3 | 1.683e-012 | 5.435e-007 | 0.003 | |

Data were obtained by the UAA=Tyr/Gln UGA= Trp and UAG=Tyr codification

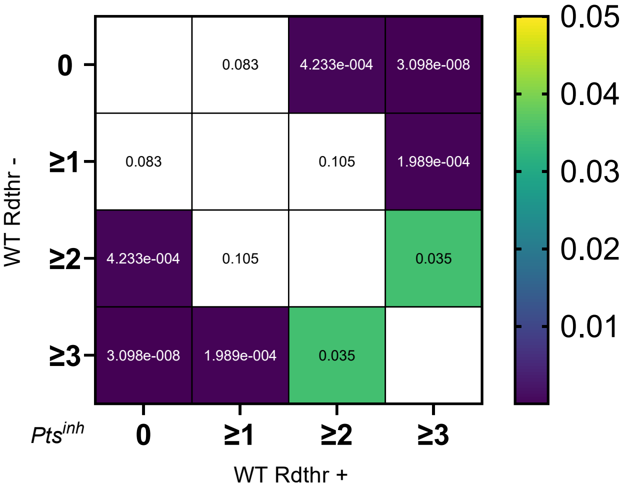
**A** Pts $^{inh=0}$, all patients for whom inhibitor was not reported or was reported as negative;

**B** Pts $^{inh=0}$ only patients for whom the absence of inhibitor was reported in the database

## Supplementary Table S3

### Distribution of Pts$^{inh}$ based on WT readthrough (UAG=Gln).

| *WT Rdthr* | *0* | *≥1* | *≥2* | *≥3* |
|---|---|---|---|---|
| + | 209 | 46 | 22 | 8 |
| *(%)* | *(25.6)* | *(19.9)* | *(13.3)* | *(5.9)* |
| - | 608 | 185 | 144 | 128 |
| *Total* | *817* | *231* | *166* | *136* |

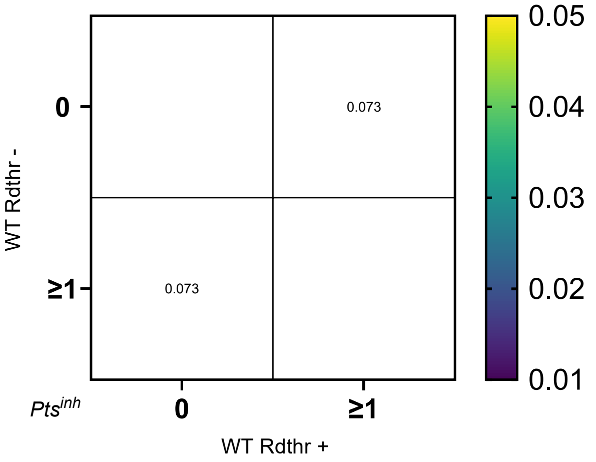


Data were obtained by the UAA=Tyr/Gln UGA= Trp  and UAG=Gln codification

## Supplementary Table S4

### WT readthrough among Pts$^{inh=0}$ and Pts$^{inh\geq1}$ after implementation with CHAMP database data

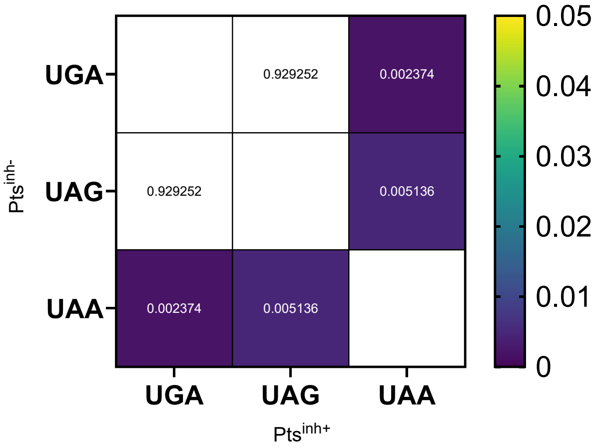
| *WT Rdthr* | *0* | *≥1* |
|---|---|---|
| + | 159 | 35 |
| *(%)* | *(19.7)* | *(14.5)* |
| - | 648 | 206 |
| *Total* | *807* | *241* |



## Supplementary Table S5

## Distribution of Pts$^{inh}$ for each PTC class.

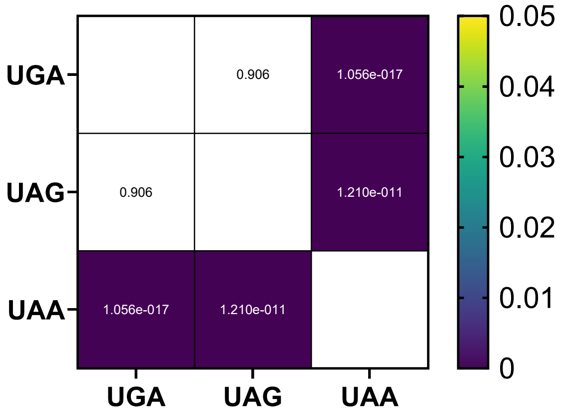
| *Inh* | *UGA* | *UAG* | *UAA* | *Total* |
|---|---|---|---|---|
| +<br>*(%)* | *146*<br>*(23.8)* | *59*<br>*(24.3)* | *26*<br>*(13.5)* | 231 |
| - | *467* | *184* | *166* | 817 |
| *Total* | *613* | *243* | *192* | 1048 |



## Supplementary Table S6

## Distribution for each PTC class of Pts with PTCs undergoing WT Rdthr

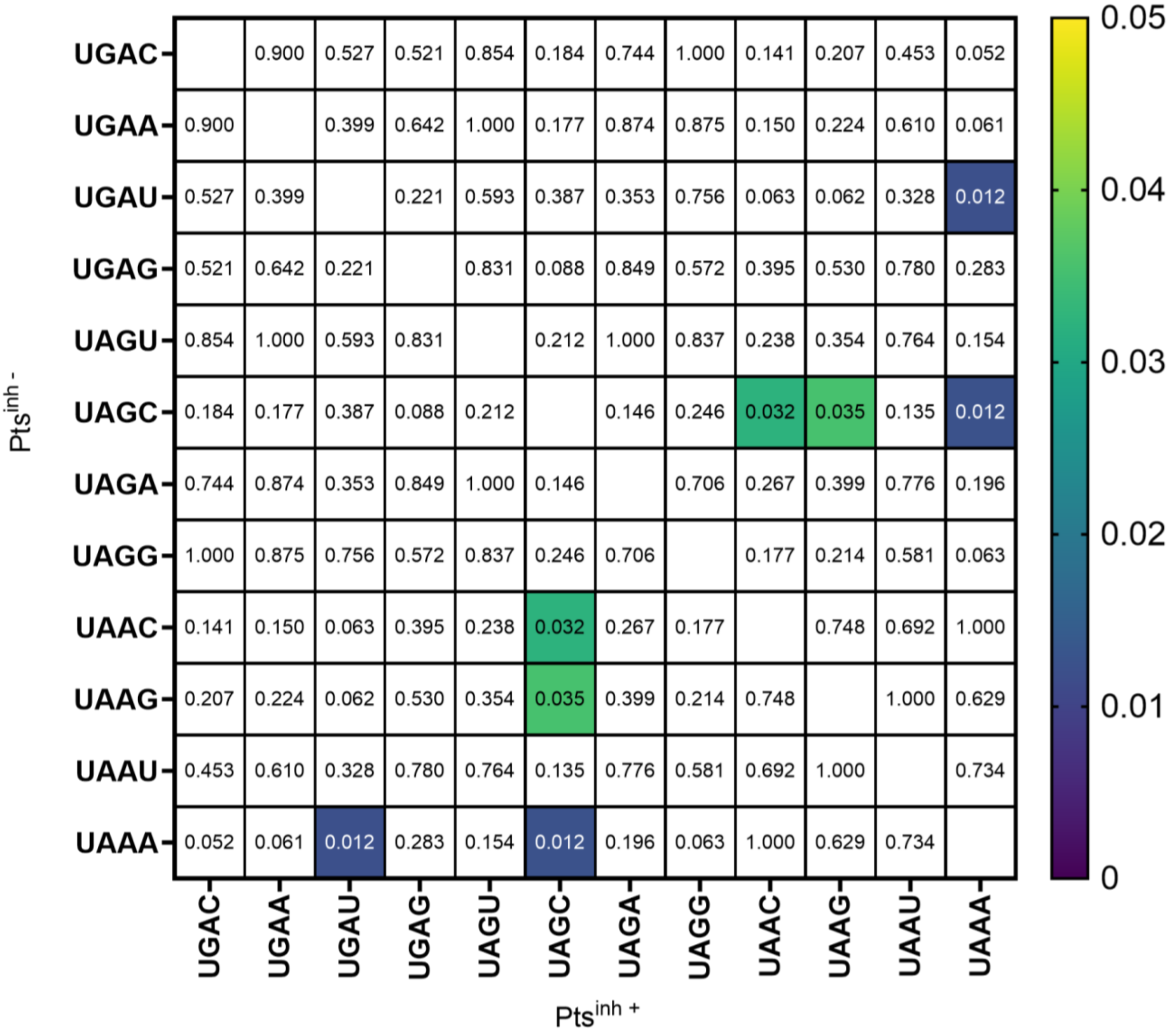
| *WT Rdthr* | *UGA* | *UAG* | *UAA* | *Total* |
|---|---|---|---|---|
| +<br>*(%)* | *69*<br>*(11.2)* | *28*<br>*(11.5)* | *98*<br>*(51.0)* | 195 |
| *Total* | *613* | *243* | *192* | 1048 |



## Supplementary Table S7

## Distribution of $Pts^{inh}$ for each tetranucleotide class.

| Inh | UGAC | UGAA | UGAU | UGAG | UAGU | UAGC | UAGA | UAGG | UAAC | UAAG | UAAU | UAAA | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | 37 | 46 | 46 | 17 | 12 | 11 | 17 | 19 | 3 | 10 | 4 | 9 | 231 |
| (%) | (24) | (23) | (27) | (19) | (22) | (35) | (21) | (24) | (10) | (15) | (16) | (12) | |
| - | 118 | 155 | 124 | 70 | 42 | 20 | 63 | 59 | 26 | 55 | 21 | 64 | 817 |
| Total | 155 | 201 | 170 | 87 | 54 | 31 | 80 | 78 | 29 | 65 | 25 | 73 | 1048 |

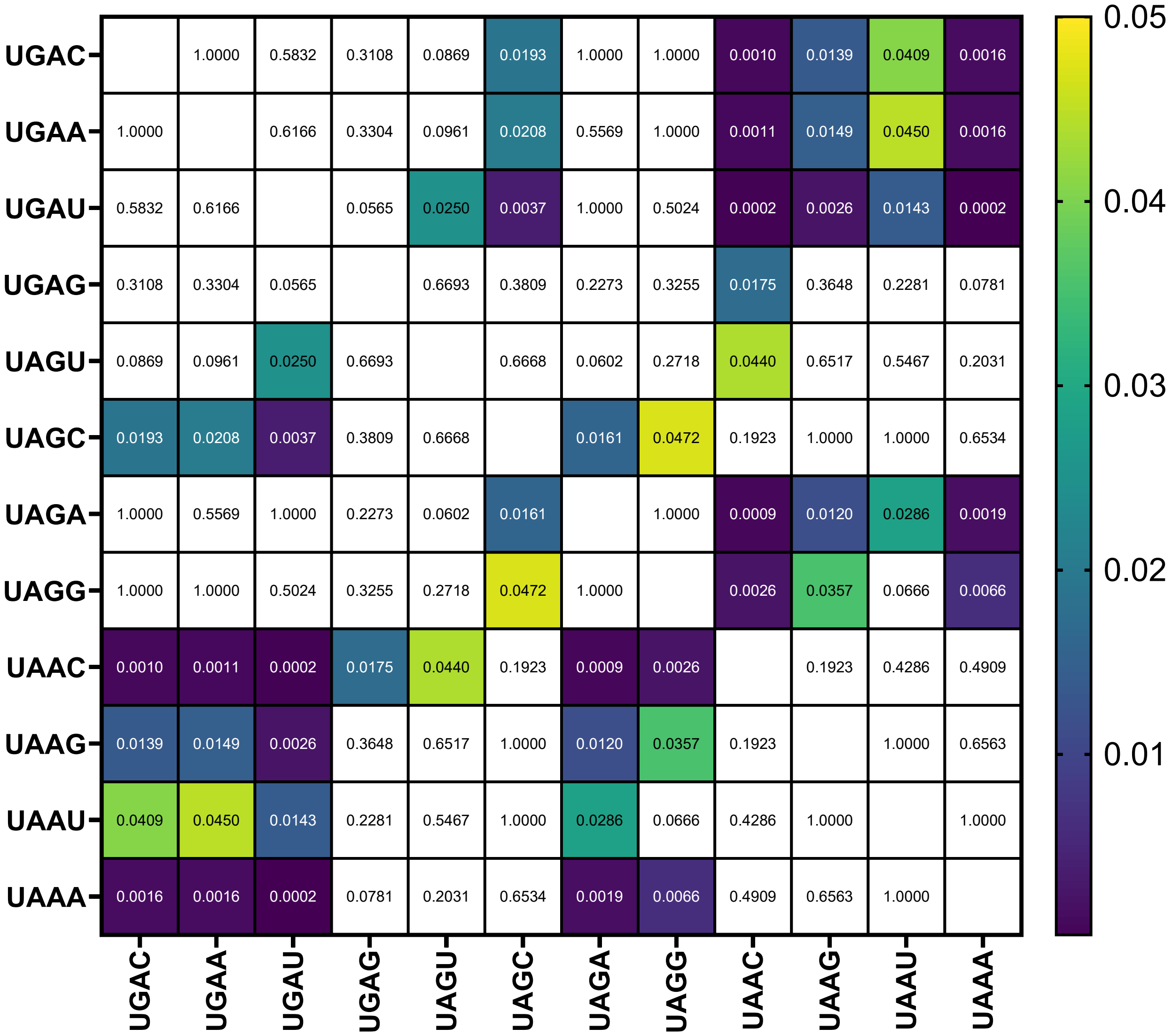


| $Pts^{inh\ -}$ \ $Pts^{inh\ +}$ | UGAC | UGAA | UGAU | UGAG | UAGU | UAGC | UAGA | UAGG | UAAC | UAAG | UAAU | UAAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UGAC | | 0.900 | 0.527 | 0.521 | 0.854 | 0.184 | 0.744 | 1.000 | 0.141 | 0.207 | 0.453 | 0.052 |
| UGAA | 0.900 | | 0.399 | 0.642 | 1.000 | 0.177 | 0.874 | 0.875 | 0.150 | 0.224 | 0.610 | 0.061 |
| UGAU | 0.527 | 0.399 | | 0.221 | 0.593 | 0.387 | 0.353 | 0.756 | 0.063 | 0.062 | 0.328 | 0.012 |
| UGAG | 0.521 | 0.642 | 0.221 | | 0.831 | 0.088 | 0.849 | 0.572 | 0.395 | 0.530 | 0.780 | 0.283 |
| UAGU | 0.854 | 1.000 | 0.593 | 0.831 | | 0.212 | 1.000 | 0.837 | 0.238 | 0.354 | 0.764 | 0.154 |
| UAGC | 0.184 | 0.177 | 0.387 | 0.088 | 0.212 | | 0.146 | 0.246 | 0.032 | 0.035 | 0.135 | 0.012 |
| UAGA | 0.744 | 0.874 | 0.353 | 0.849 | 1.000 | 0.146 | | 0.706 | 0.267 | 0.399 | 0.776 | 0.196 |
| UAGG | 1.000 | 0.875 | 0.756 | 0.572 | 0.837 | 0.246 | 0.706 | | 0.177 | 0.214 | 0.581 | 0.063 |
| UAAC | 0.141 | 0.150 | 0.063 | 0.395 | 0.238 | 0.032 | 0.267 | 0.177 | | 0.748 | 0.692 | 1.000 |
| UAAG | 0.207 | 0.224 | 0.062 | 0.530 | 0.354 | 0.035 | 0.399 | 0.214 | 0.748 | | 1.000 | 0.629 |
| UAAU | 0.453 | 0.610 | 0.328 | 0.780 | 0.764 | 0.135 | 0.776 | 0.581 | 0.692 | 1.000 | | 0.734 |
| UAAA | 0.052 | 0.061 | 0.012 | 0.283 | 0.154 | 0.012 | 0.196 | 0.063 | 1.000 | 0.629 | 0.734 | |

## Supplementary Table S8

## Distribution of Pts with WT Rdthr for each tetranucleotide class.

| WT Rdthr | UGAC | UGAA | UGAU | UGAG | UAGU | UAGC | UAGA | UAGG | UAAC | UAAG | UAAU | UAAA | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | 2 | 3 | 1 | 3 | 3 | 4 | 0 | 1 | 3 | 4 | 2 | 5 | 31 |
| (%) | (5.4) | (6.5) | (2.2) | (18) | (25) | (36) | (0.0) | (5.3) | (100) | (40) | (50) | (55.6) | |
| - | 35 | 43 | 45 | 14 | 9 | 7 | 17 | 18 | 0 | 6 | 2 | 4 | 200 |
| Total | 37 | 46 | 46 | 17 | 12 | 11 | 17 | 19 | 3 | 10 | 4 | 9 | 231 |



| | UGAC | UGAA | UGAU | UGAG | UAGU | UAGC | UAGA | UAGG | UAAC | UAAG | UAAU | UAAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UGAC | | 1.0000 | 0.5832 | 0.3108 | 0.0869 | 0.0193 | 1.0000 | 1.0000 | 0.0010 | 0.0139 | 0.0409 | 0.0016 |
| UGAA | 1.0000 | | 0.6166 | 0.3304 | 0.0961 | 0.0208 | 0.5569 | 1.0000 | 0.0011 | 0.0149 | 0.0450 | 0.0016 |
| UGAU | 0.5832 | 0.6166 | | 0.0565 | 0.0250 | 0.0037 | 1.0000 | 0.5024 | 0.0002 | 0.0026 | 0.0143 | 0.0002 |
| UGAG | 0.3108 | 0.3304 | 0.0565 | | 0.6693 | 0.3809 | 0.2273 | 0.3255 | 0.0175 | 0.3648 | 0.2281 | 0.0781 |
| UAGU | 0.0869 | 0.0961 | 0.0250 | 0.6693 | | 0.6668 | 0.0602 | 0.2718 | 0.0440 | 0.6517 | 0.5467 | 0.2031 |
| UAGC | 0.0193 | 0.0208 | 0.0037 | 0.3809 | 0.6668 | | 0.0161 | 0.0472 | 0.1923 | 1.0000 | 1.0000 | 0.6534 |
| UAGA | 1.0000 | 0.5569 | 1.0000 | 0.2273 | 0.0602 | 0.0161 | | 1.0000 | 0.0009 | 0.0120 | 0.0286 | 0.0019 |
| UAGG | 1.0000 | 1.0000 | 0.5024 | 0.3255 | 0.2718 | 0.0472 | 1.0000 | | 0.0026 | 0.0357 | 0.0666 | 0.0066 |
| UAAC | 0.0010 | 0.0011 | 0.0002 | 0.0175 | 0.0440 | 0.1923 | 0.0009 | 0.0026 | | 0.1923 | 0.4286 | 0.4909 |
| UAAG | 0.0139 | 0.0149 | 0.0026 | 0.3648 | 0.6517 | 1.0000 | 0.0120 | 0.0357 | 0.1923 | | 1.0000 | 0.6563 |
| UAAU | 0.0409 | 0.0450 | 0.0143 | 0.2281 | 0.5467 | 1.0000 | 0.0286 | 0.0666 | 0.4286 | 1.0000 | | 1.0000 |
| UAAA | 0.0016 | 0.0016 | 0.0002 | 0.0781 | 0.2031 | 0.6534 | 0.0019 | 0.0066 | 0.4909 | 0.6563 | 1.0000 | |

## Supplementary Table S9

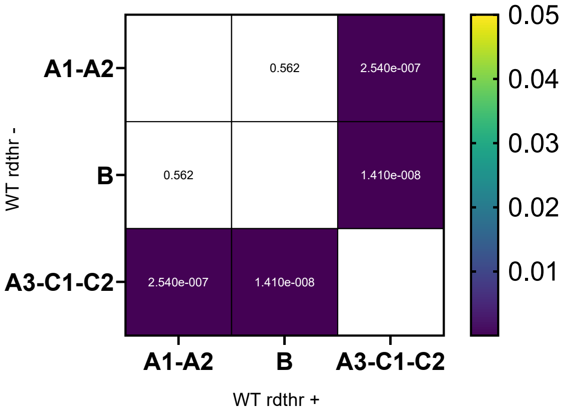
## Distribution of Pts$^{inh}$ for domain localization of PTCs and for PTC classes.

| | *All PTC* | | | | *UGA* | | | *UAG* | | | *UAA* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Inh* | *A1-A2* | *B* | *A3-C1-C2* | *Total* | *A1-A2* | *B* | *A3-C1-C2* | *A1-A2* | *B* | *A3-C1-C2* | *A1-A2* | *B* | *A3-C1-C2* |
| + | *42* | *34* | *157* | *230* | *24* | *15* | *106* | *14* | *10* | *35* | *4* | *9* | *13* |
| *(%)* | *(14.7)* | *(12.9)* | *(32.1)* | *(22.4)* | *(14.8)* | *(15.6)* | *(31.6)* | *(17.9)* | *(15.9)* | *(34.3)* | *(8.7)* | *(8.6)* | *(30.9)* |
| - | *244* | *229* | *322* | *795* | *138* | *81* | *229* | *64* | *53* | *67* | *42* | *95* | *29* |
| *Total* | *286* | *263* | *479* | *1028* | *162* | *96* | *335* | *78* | *63* | *102* | *46* | *104* | *42* |

## Supplementary Table S10

## Distribution of Pts WT Rdthr based on FVIII domain localization of PTCs.

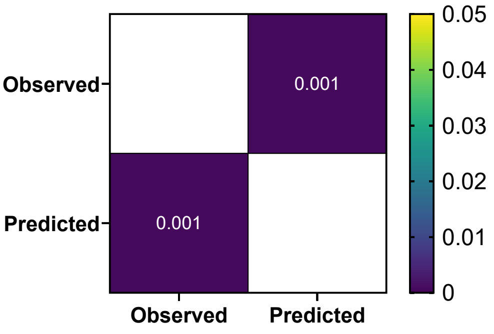
| *WT Rdthr* | *A1-A2* | *B* | *A3-C1-C2* | *Total* |
|---|---|---|---|---|
| + | *72* | *72* | *51* | 195 |
| *(%)* | *(25.2)* | *(27.4)* | *(10.6)* | |
| - | *214* | *191* | *428* | 833 |
| *Total* | *286* | *263* | *479* | 1028 |



## Supplementary Table S11

## Distribution of WT Rdthr for PTCs observed (EAHAD) and predicted (never reported) as single nucleotide variants.

| *WT Rdthr* | *Observed* | *Predicted* | *Total* |
|---|---|---|---|
| + | *105* | *144* | *249* |
| *(%)* | *(31)* | *(22)* | *(25)* |
| - | *230* | *518* | *748* |
| *Total* | *335* | *662* | *997* |



## Supplementary Table S12

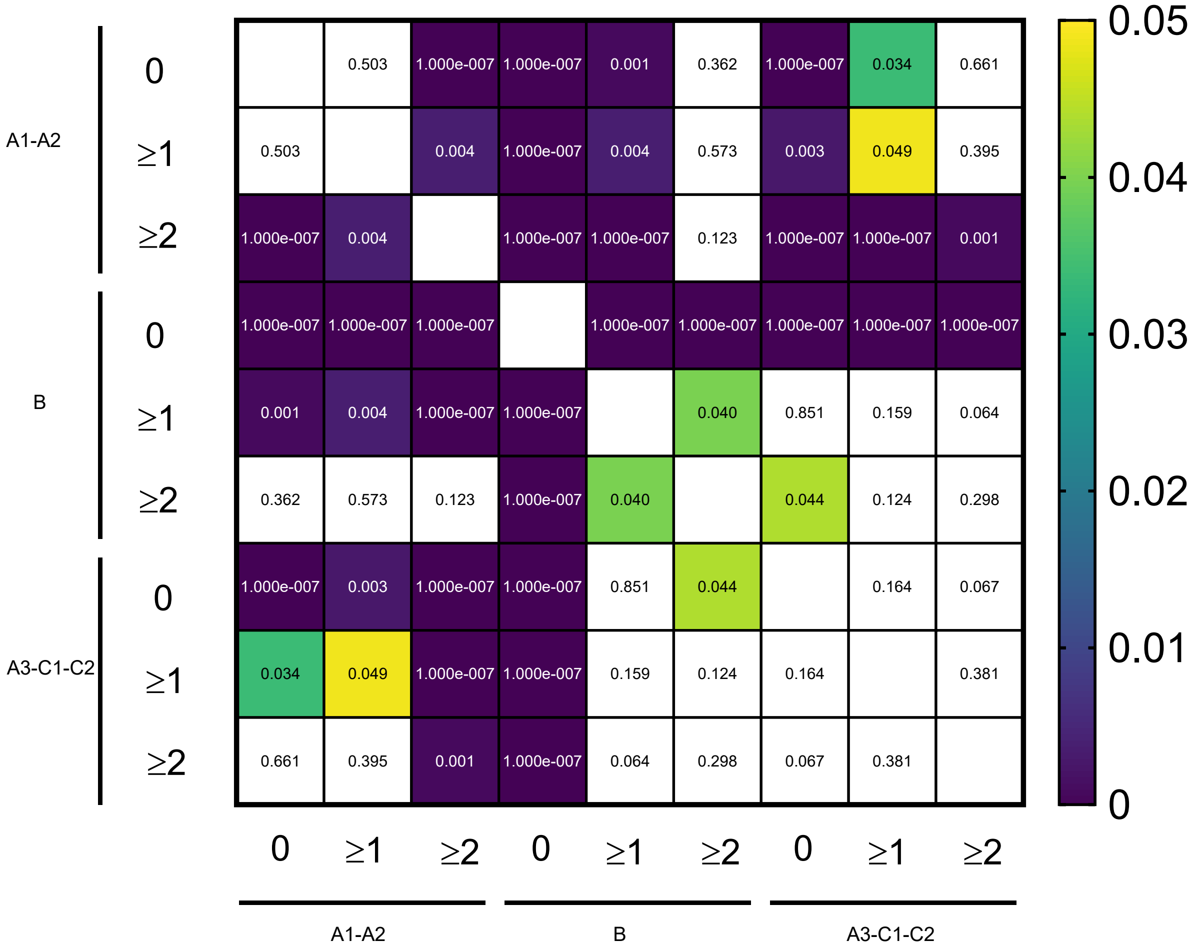
**Distribution for each PTC class of PTCs observed (EAHAD) and predicted (never reported) as single nucleotide variants.**

| | *Observed n(%)* | *Predicted n(%)* | *Total n(%)* |
|---|---|---|---|
| *UGA* | *88(27)* | *160(24)* | *248(25)* |
| *UAG* | *122(36)* | *223(34)* | *345(35)* |
| *UAA* | *125(37)* | *279(42)* | *404(41)* |
| *Total* | *335* | *662* | *997* |

## Supplementary Table S13

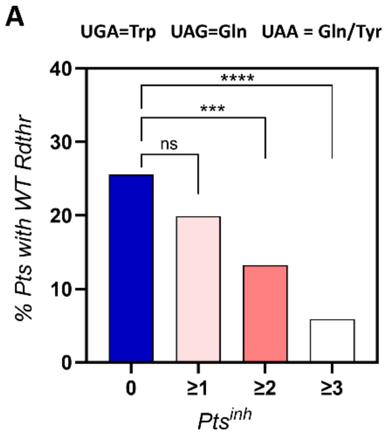
## Mean differential affinity, expressed as percentile ranking, of peptides obtained from WT Rdthr- PTCs grouped by $PTC^{inh}$ status and FVIII domain location.

|  | *A1-A2* |  |  | *B* |  |  | *A3-C1-C2* |  |  |
|---|---|---|---|---|---|---|---|---|---|
| $PTC^{inh}$ | 0 | ≥1 | ≥2 | 0 | ≥1 | ≥2 | 0 | ≥1 | ≥2 |
| **Mean** | 0,53 | 0,80 | 3,00 | -2,25 | -0,55 | 1,32 | -0,48 | -0,04 | 0,34 |
| **Standard error** | 0,16 | 0,37 | 0,66 | 0,19 | 0,29 | 0,86 | 0,23 | 0,21 | 0,38 |
| **Count** | 1768 | 364 | 156 | 2574 | 728 | 104 | 1144 | 1118 | 442 |
| **Variants number** | 68 | 14 | 6 | 99 | 28 | 4 | 44 | 43 | 17 |



|  |  | A1-A2 0 | A1-A2 ≥1 | A1-A2 ≥2 | B 0 | B ≥1 | B ≥2 | A3-C1-C2 0 | A3-C1-C2 ≥1 | A3-C1-C2 ≥2 |
|---|---|---|---|---|---|---|---|---|---|---|
| A1-A2 | 0 |  | 0.503 | 1.000e-007 | 1.000e-007 | 0.001 | 0.362 | 1.000e-007 | 0.034 | 0.661 |
|  | ≥1 | 0.503 |  | 0.004 | 1.000e-007 | 0.004 | 0.573 | 0.003 | 0.049 | 0.395 |
|  | ≥2 | 1.000e-007 | 0.004 |  | 1.000e-007 | 1.000e-007 | 0.123 | 1.000e-007 | 1.000e-007 | 0.001 |
| B | 0 | 1.000e-007 | 1.000e-007 | 1.000e-007 |  | 1.000e-007 | 1.000e-007 | 1.000e-007 | 1.000e-007 | 1.000e-007 |
|  | ≥1 | 0.001 | 0.004 | 1.000e-007 | 1.000e-007 |  | 0.040 | 0.851 | 0.159 | 0.064 |
|  | ≥2 | 0.362 | 0.573 | 0.123 | 1.000e-007 | 0.040 |  | 0.044 | 0.124 | 0.298 |
| A3-C1-C2 | 0 | 1.000e-007 | 0.003 | 1.000e-007 | 1.000e-007 | 0.851 | 0.044 |  | 0.164 | 0.067 |
|  | ≥1 | 0.034 | 0.049 | 1.000e-007 | 1.000e-007 | 0.159 | 0.124 | 0.164 |  | 0.381 |
|  | ≥2 | 0.661 | 0.395 | 0.001 | 1.000e-007 | 0.064 | 0.298 | 0.067 | 0.381 |  |

## Supplementary Figure S1



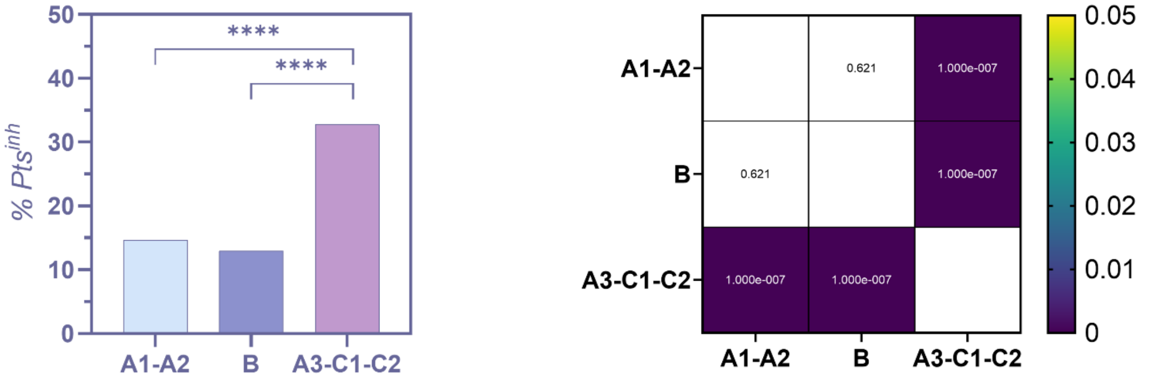
**B**

| | EAHAD (UAG=Tyr) | EAHAD (UAG=Gln) |
|---|---|---|
| 0 vs ≥1 | 2,16E-02 | 8,25E-02 |
| 0 vs ≥2 | 3,45E-06 | 4,23E-04 |
| 0 vs ≥3 | 1,11E-12 | 3,10E-08 |
| ≥2 vs ≥3 | 2,50E-03 | 3,50E-02 |
| ≥1 vs ≥3 | 5,44E-07 | 1,99E-04 |

**Insertion of WT amino acid upon readthrough in the UAG=Gln model**

A) HA patients with *F8* PTCs predicted to undergo WT readthrough, exploring Gln at UAG, were classified in accordance with PTCs associated with an increasing number of inhibitor-positive cases ($\text{Pts}^{\text{inh=0}}$, patients with PTCs never found in patients with inhibitors; $\text{Pts}^{\text{inh}\geq 1}$,$\text{Pts}^{\text{inh}\geq 2}$ $\text{Pts}^{\text{inh}\geq 3}$ , patients affected by PTCs found in 1,2,3 or more patients with inhibitors). B) Statistical comparison between models with Tyr or Gln reinsertion at UAG. Statistical significance of Fisher’s exact test.

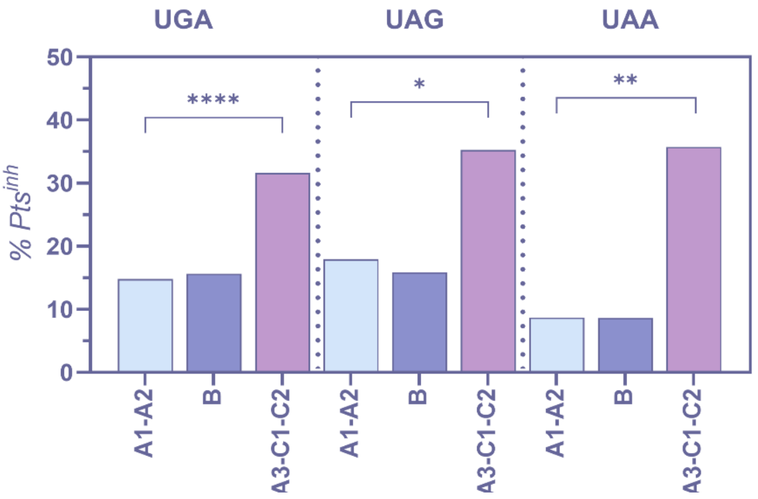
## Supplementary Figure S2



**Comparison of the percentage of Pts$^{inh}$ with PTCs in FVIII domains.**

*Left panel*. Distribution of Pts$^{inh}$ with PTCs in grouped FVIII domains. *Right panel*. Statistical Comparison

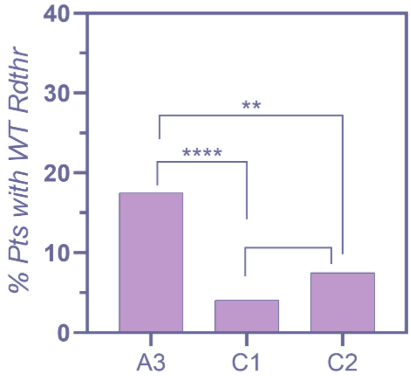
## Supplementary Figure S3



**Comparison of the percentage of Pts$^{inh}$ for PTCs in FVIII domains.**

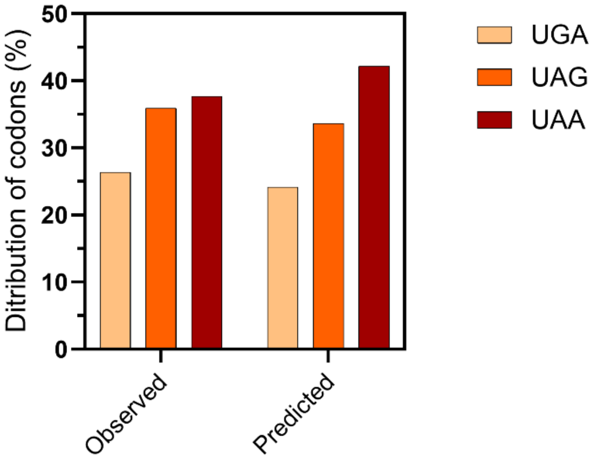
Distribution of Pts$^{inh}$ in relation to FVIII domain location of PTC classes (UGA/UAG/UAA).

## Supplementary Figure S4



**Comparison of the percentage of Pts with PTCs with WT Rdthr in single FVIII domains (A3, C1 and C2)**

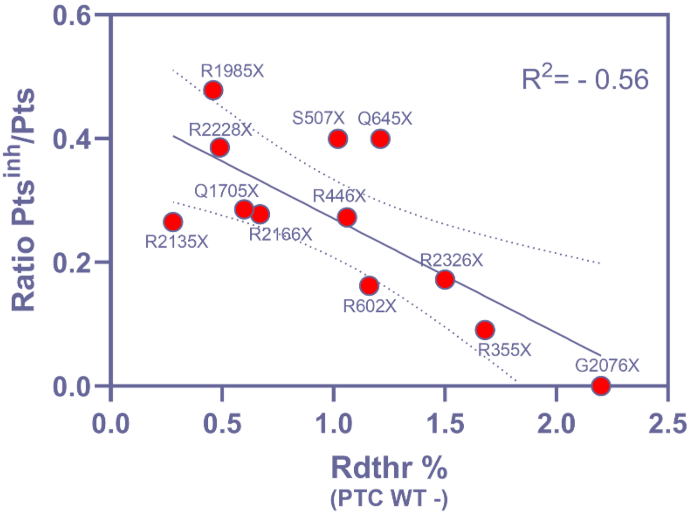
## Supplementary Figure S5



**Comparison of observed (EAHAD) and predicted (never reported before) PTCs for each PTC class (UGA, UAG, UAA).**

Prediction of PTCs produced by single nucleotide variants (SNV).

## Supplementary Figure S6



**Correlation between recombinant Rdthr output and ratio of patients with inhibitor on total number of patients for WT Rdthr- PTCs.**

Inverse relation between readthrough over PTCs WT –, excluding those within B domain, and ratio of patients with inhibitor on total number of patients for 12 PTCs WT – (red dots).