|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mutant** | ***Kcat* lit** | **(+/-)** | **kcat dark** | **(+/-)** | **Km**  **lit** | **(+/-)** | **Km dark** | **(+/-)** | ***kcat*/Km**  **lit** | | **(+/-)** | ***kcat*/Km dark** | **(+/-)** |
| Unmutated DL121 | 0.244 | 0.006 | 0.190 | 0.006 | 0.826 | 0.107 | 1.186 | 0.174 | 0.295 | 0.039 | | 0.160 | 0.024 |
| DL121 C450S | 0.221 | 0.007 | 0.197 | 0.006 | 0.822 | 0.141 | 0.833 | 0.128 | 0.269 | 0.047 | | 0.236 | 0.037 |
| DL121 A9N | 0.824 | 0.027 | 0.566 | 0.024 | 1.896 | 0.318 | 1.311 | 0.337 | 0.435 | 0.074 | | 0.432 | 0.113 |
| DL121 M16A | 0.241 | 0.005 | 0.134 | 0.006 | 0.972 | 0.118 | 1.036 | 0.258 | 0.247 | 0.030 | | 0.129 | 0.033 |
| DL121 R52K | 0.291 | 0.011 | 0.153 | 0.007 | 0.961 | 0.224 | 1.127 | 0.321 | 0.303 | 0.071 | | 0.135 | 0.039 |
| DL121 D87A | 0.334 | 0.011 | 0.173 | 0.005 | 0.724 | 0.138 | 0.627 | 0.115 | 0.462 | 0.089 | | 0.276 | 0.051 |
| DL121 E120P | 0.491 | 0.015 | 0.278 | 0.009 | 1.385 | 0.205 | 0.833 | 0.157 | 0.354 | 0.054 | | 0.333 | 0.064 |
| DL121 D122W | 0.124 | 0.003 | 0.106 | 0.003 | 1.238 | 0.174 | 1.719 | 0.269 | 0.100 | 0.014 | | 0.061 | 0.010 |
| DL121 H124Q | 0.291 | 0.010 | 0.144 | 0.005 | 1.118 | 0.193 | 1.003 | 0.167 | 0.260 | 0.046 | | 0.144 | 0.024 |
| DL121 D127W | 0.288 | 0.009 | 0.171 | 0.005 | 0.388 | 0.088 | 0.857 | 0.136 | 0.741 | 0.170 | | 0.199 | 0.032 |
| DL121 M16A,H124Q | 0.202 | 0.003 | 0.063 | 0.003 | 0.683 | 0.136 | 0.153 | 0.043 | 0.295 | 0.059 | | 0.409 | 0.116 |
| DL121- M16A,D87A,H124Q | 0.171 | 0.003 | 0.044 | 0.002 | 0.828 | 0.193 | 0.519 | 0.135 | 0.207 | 0.048 | | 0.084 | 0.022 |

**Supplementary File 1a**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cutoff for sector definition:** | 0.005 | 0.008 | 0.01 | 0.015 |
| Inactivating mutations in sector | 223 | 332 | 373 | 448 |
| Expected by chance | 177 | 280 | 311 | 381 |
| p-value: | 5.73 x 10-7 | 2.34 x 10-6 | 7.88 x 10-8 | 3.19 x 10-8 |
|  |  |  |  |  |

**Supplementary File 1b**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cutoff for Conserved Positions:** | 1.89 | 1.54 | 1.49 | 1.38 |
| Conserved inactivating mutations | 249 | 377 | 413 | 494 |
| Expected by chance | 175 | 276 | 309 | 374 |
| p-value: | 7.26 x 10-16 | 3.78 x 10-20 | 8.70 x 10-20 | 6.60 x 10-23 |
|  |  |  |  |  |

**Supplementary File 1c**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sector Cutoff:** | 0.005 | 0.008 | 0.01 | 0.015 |
| **Cutoff for allostery enhancing:** | Allosteric positions in sector: | 4 | 5 | 5 | 7 |
| Expected | 13 | 22 | 25 | 31 |
| p < 0.05 | p-value: | 0.008 | 3.43 x 10-5 | 5.82 x 10-6 | 3.52 x 10-7 |
| p < 0.016 | # in sector: | 0 | 0 | 0 | 1 |
| Expected | 6 | 11 | 12 | 15 |
| p-value: | 0.013 | 4.23 x 10-4 | 1.68 x 10-4 | 4.64 x 10-5 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | **Sector Cutoff:** | 0.005 | 0.008 | 0.01 | 0.015 |
| **Cutoff for allostery disrupting:** | Allosteric positions in sector: | 2 | 15 | 15 | 15 |
| Expected | 5 | 8 | 9 | 11 |
| p < 0.05 | p-value: | 0.257 | 0.013 | 0.038 | 0.25 |
| p < 0.016 | # in sector: | 1 | 7 | 7 | 7 |
| Expected | 1 | 2 | 3 | 3 |
| p-value: | 0.967 | 0.004 | 0.010 | 0.049 |

**Supplementary File 1d**

|  |  |  |
| --- | --- | --- |
|  | **Allosteric Surface Positions** | |
| **Cutoff for allostery enhancing:** | Observed | 88 |
| Expected | 73 |
| p < 0.05 | p-value: | 0.004 |
| p < 0.016 | Observed | 44 |
| Expected | 35 |
| p-value: | 0.013 |
|  |  |  |
|  |  |  |
|  | **Allosteric Surface Positions** | |
| **Cutoff for allostery disrupting:** | Observed | 33 |
| Expected | 27 |
| p < 0.05 | p-value: | 0.061 |
| p < 0.016 | Observed | 12 |
| Expected | 8 |
| p-value: | 0.048 |

**Supplementary File 1e**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sector Cutoff:** | 0.005 | 0.008 | 0.01 | 0.015 |
| **Cutoff for allostery enhancing:** | Allosteric surface positions within or connected to sector: | 2 | 13 | 15 | 28 |
| Expected | 18 | 27 | 28 | 33 |
| p < 0.05 | p-value: | 4.88 x 10-5 | 0.002 | 0.006 | 0.360 |
| p < 0.016 | # connected: | 10 | 5 | 7 | 15 |
| Expected | 1 | 13 | 13 | 16 |
| p-value: | 0.009 | 0.019 | 0.074 | 0.987 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | **Sector Cutoff:** | 0.005 | 0.008 | 0.01 | 0.015 |
| **Cutoff for allostery disrupting:** | Allosteric surface positions within or connected to sector: | 19 | 22 | 22 | 24 |
| Expected | 6 | 10 | 10 | 12 |
| p < 0.05 | p-value: | 1.56 x 10-7 | 1.52 x 10-5. | 2.72 x 10-5. | 6.60 x 10-5 |
| p < 0.016 | # connected: | 8 | 9 | 9 | 10 |
| Expected | 2 | 3 | 3 | 4 |
| p-value: | 1.38 x 10-5 | 2.30 x 10-4 | 3.16 x 10-4 | 2.50 x 10-4 |

**Supplementary File 1f**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sector Cutoff:** | 0.005 | 0.008 | 0.01 | 0.015 |
| **Cutoff for allostery enhancing:** | Allosteric surface positions connected to sector: | 2 | 13 | 15 | 28 |
| Expected | 13 | 18 | 17 | 19 |
| p < 0.05 | p-value: | 0.001 | 0.269 | 0.697 | 0.033 |
| p < 0.016 | # connected: | 1 | 5 | 7 | 15 |
| Expected | 6 | 8 | 8 | 9 |
| p-value: | 0.041 | 0.275 | 0.837 | 0.049 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | **Sector Cutoff:** | 0.005 | 0.008 | 0.01 | 0.015 |
| **Cutoff for allostery disrupting:** | Allosteric surface positions connected to sector: | 18 | 7 | 7 | 9 |
| Expected | 5 | 6 | 6 | 7 |
| p < 0.05 | p-value: | 3.83 x 10-10 | 0.971 | 0.883 | 0.379 |
| p < 0.016 | # connected: | 8 | 2 | 2 | 3 |
| Expected | 1 | 2 | 2 | 2 |
| p-value: | 8.02 x 10-8 | 0.731 | 0.772 | 0.777 |

**Supplementary File 1g**