### Functional Mapping of Protein-Protein Interactions in an Enzyme Complex by Directed Evolution
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### Supporting Table S1  Compilation of amino acids observed in MtCM variants before and after selection (Fig. 5A-C tabular version)

#### (A) Amino acid residues found under non-selective conditions (M9c +FY)

| Wt Residue | Position G84 found / library size in % | Position R85 found / library size in % | Position G86 found / library size in % | Position R87 found / library size in % | Position L88 found / library size in % | Position G89 found / library size in % | Position H90 found / library size in % |
|------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| G          | 5/41                                 | 6/35                                 | 5/39                                 | 18/34                                | 9/34                                 | 3/34                                 |
| A          | 6/32                                 | 3/35                                 | 8/39                                 | 3/31                                 | 15/34                                | 12/34                                |
| S          | 6/32                                 | 3/35                                 | 5/39                                 | 13/34                                | 9/34                                 | 3/34                                 |
| C          | 6/32                                 | 5/35                                 | 6/39                                 | 6/34                                 | 3/34                                 |
| T          | 6/32                                 | 6/35                                 | 2/39                                 | 9/34                                 | 3/34                                 |
| D          | 6/32                                 | 8/35                                 | 5/39                                 | 9/34                                 |
| P          | 6/32                                 | 11/35                                | 6/39                                 | 6/34                                 |
| N          | 7/32                                 | 6/35                                 | 10/39                                | 3/34                                 |
| V          | 5/32                                 | 9/35                                 | 5/39                                 | 10/34                                |
| E          | 6/32                                 | 1/35                                 | 3/39                                 | 3/34                                 |
| Q          | 2/32                                 | 10/39                                | 3/34                                 |
| H          | 2/32                                 | 1/35                                 | 15/34                                |
| L          | 10/32                                | 3/39                                 | 6/34                                 |
| I          | 6/32                                 | 14/35                                | 9/34                                 |
| M          | 3/32                                 | 3/39                                 | 3/34                                 |
| K          | 2/32                                 | 6/35                                 | 3/34                                 |
| R          | 7/32                                 | 5/39                                 | 3/34                                 |
| F          | 7/32                                 | 6/35                                 | 3/34                                 |
| Y          | 3/32                                 | 5/39                                 | 3/34                                 |
| W          | 2/32                                 | 3/34                                 | 3/34                                 |
(B) Preferred residues selected under a stringent regime (M9c, no inducer present) in the presence of MtDS (pKIMP-ACG)

| Wt Residue | Position G84 found / library size in % | Position R85 found / library size in % | Position G86 found / library size in % | Position R87 found / library size in % | Position L88 found / library size in % | Position G89 found / library size in % | Position H90 found / library size in % |
|------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| G          | 73                                   | 33/45                                | 100                                  | 1/89                                 | 51                                   | 40/78                                | 23                                   | 13/57                                |
| A          | 18                                   | 8/45                                 | 10                                   | 9/89                                 | 3                                    | 2/78                                 | 4                                    | 2/57                                 |
| S          | 4                                    | 2/45                                 | 12                                   | 11/89                                | 3                                    | 2/66                                 | 10                                   | 8/78                                 | 7                                    | 4/57                                 |
| C          |                                      |                                      | 12                                   | 2/66                                 |                                       |                                      |                                       |                                      |
| T          | 4                                    | 2/45                                 | 8                                    | 7/89                                 | 2                                    | 1/66                                 | 11                                   | 6/57                                 |
| D          |                                      |                                      |                                      |                                      |                                       |                                       |                                       |                                      |
| P          | 7                                    | 6/89                                 | 11                                   | 7/66                                 | 1                                    | 1/78                                 | 2                                    | 1/57                                 |
| N          | 8                                    | 7/89                                 |                                      |                                      |                                       |                                       |                                       |                                      |
| V          | 4                                    | 4/89                                 | 5                                    | 3/66                                 | 3                                    | 2/78                                 | 4                                    | 2/57                                 |
| E          | 1                                    | 1/89                                 |                                      |                                      |                                       |                                       |                                       |                                      |
| Q          | 3                                    | 3/89                                 |                                      |                                      |                                       |                                       |                                       |                                      |
| H          | 4                                    | 4/89                                 |                                      |                                      |                                       |                                       |                                       |                                      |
| L          | 10                                   | 9/89                                 | 62                                   | 41/66                                | 2                                    | 1/57                                 |                                       |                                      |
| I          | 1                                    | 1/89                                 | 8                                    | 5/66                                 | 2                                    | 1/57                                 |                                       |                                      |
| M          |                                      |                                      |                                      |                                      |                                       |                                       |                                       |                                      |
| K          | 9                                    | 8/89                                 |                                      |                                      |                                       |                                       |                                       |                                      |
| R          | 100                                  | 83/83                                | 19                                   | 17/89                                | 6                                    | 5/78                                 | 21                                   | 12/57                                |
| F          |                                      |                                      |                                      |                                      |                                       |                                       |                                       |                                      |
| Y          |                                      |                                      |                                      |                                      |                                       |                                       |                                       |                                      |
| W          |                                      |                                      |                                      |                                      |                                       |                                       |                                       |                                      |
| *          |                                      |                                      |                                      |                                      |                                       |                                       |                                       |                                      |

* Values indicate frequencies in the library.
### Residue patterns emerging in selected MtCM variants grown in the absence of MtDS (pKIMP-UAUC)

| Wt Residue | Position in % found / library size | G84 found / library size | G85 found / library size | G86 found / library size | G87 found / library size | L88 found / library size | G89 found / library size | H90 found / library size |
|------------|-----------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| G          | 17/63                             | 70/63                    | 2/62                     | 8/56                     | 3/63                     | 12/60                    | 7/60                     | 3/60                     |
| A          | 2/63                              | 8/63                     | 1/62                     | 5/65                     | 5/63                     | 3/60                     | 5/60                     | 3/60                     |
| S          | 43/63                             | 3/63                     | 11/63                    | 3/63                     | 2/63                     | 7/60                     | 3/60                     | 2/60                     |
| C          | 35/63                             | 3/63                     | 14/60                    | 3/60                     | 5/60                     | 12/60                    | 7/60                     | 8/60                     |
| T          | 10/63                             | 6/63                     | 5/63                     | 6/63                     | 2/63                     | 3/60                     | 3/60                     | 2/60                     |
| D          | 15/63                             | 1/62                     | 9/63                     | 2/63                     | 5/63                     | 3/60                     | 3/60                     | 3/60                     |
| P          | 2/63                              | 10/63                    | 3/63                     | 3/63                     | 1/63                     | 7/60                     | 4/60                     | 7/60                     |
| N          | 3/63                              | 2/63                     | 1/63                     | 2/63                     | 5/63                     | 3/60                     | 2/60                     | 2/60                     |
| V          | 10/63                             | 6/63                     | 2/63                     | 12/60                    | 5/63                     | 8/60                     | 4/60                     | 7/60                     |
| E          | 3/63                              | 2/63                     | 5/63                     | 2/63                     | 3/60                     | 1/60                     | 2/60                     | 2/60                     |
| Q          | 2/63                              | 1/63                     | 2/63                     | 8/60                     | 3/60                     | 2/60                     | 1/60                     | 2/60                     |
| H          | 5/63                              | 1/63                     | 4/63                     | 3/63                     | 2/60                     | 5/63                     | 3/60                     | 6/60                     |
| L          | 5/63                              | 3/63                     | 1/63                     | 2/63                     | 5/63                     | 10/60                    | 6/60                     | 6/60                     |
| I          | 3/63                              | 2/63                     | 2/63                     | 1/63                     | 3/60                     | 3/60                     | 3/60                     | 3/60                     |
| M          | 3/63                              | 2/63                     | 3/63                     | 5/63                     | 3/60                     | 2/60                     | 2/60                     | 2/60                     |
| K          | 7/63                              | 1/63                     | 5/63                     | 5/63                     | 3/60                     | 3/60                     | 3/60                     | 3/60                     |
| R          | 2/63                              | 1/63                     | 2/63                     | 3/63                     | 3/60                     | 3/60                     | 3/60                     | 3/60                     |
| F          | 5/63                              | 2/63                     | 3/63                     | 2/60                     | 1/60                     | 3/60                     | 2/60                     | 2/60                     |
| Y          | 5/63                              | 3/63                     | 5/63                     | 3/60                     | 3/60                     | 2/60                     | 2/60                     | 2/60                     |
| W          | 7/63                              | 4/60                     | 2/63                     | 3/60                     | 5/63                     | 3/60                     | 10/60                    | 6/60                     |
| *          | 3/63                              | 2/63                     | 2/60                     | 3/60                     | 10/60                    | 6/60                     | 6/60                     | 6/60                     |