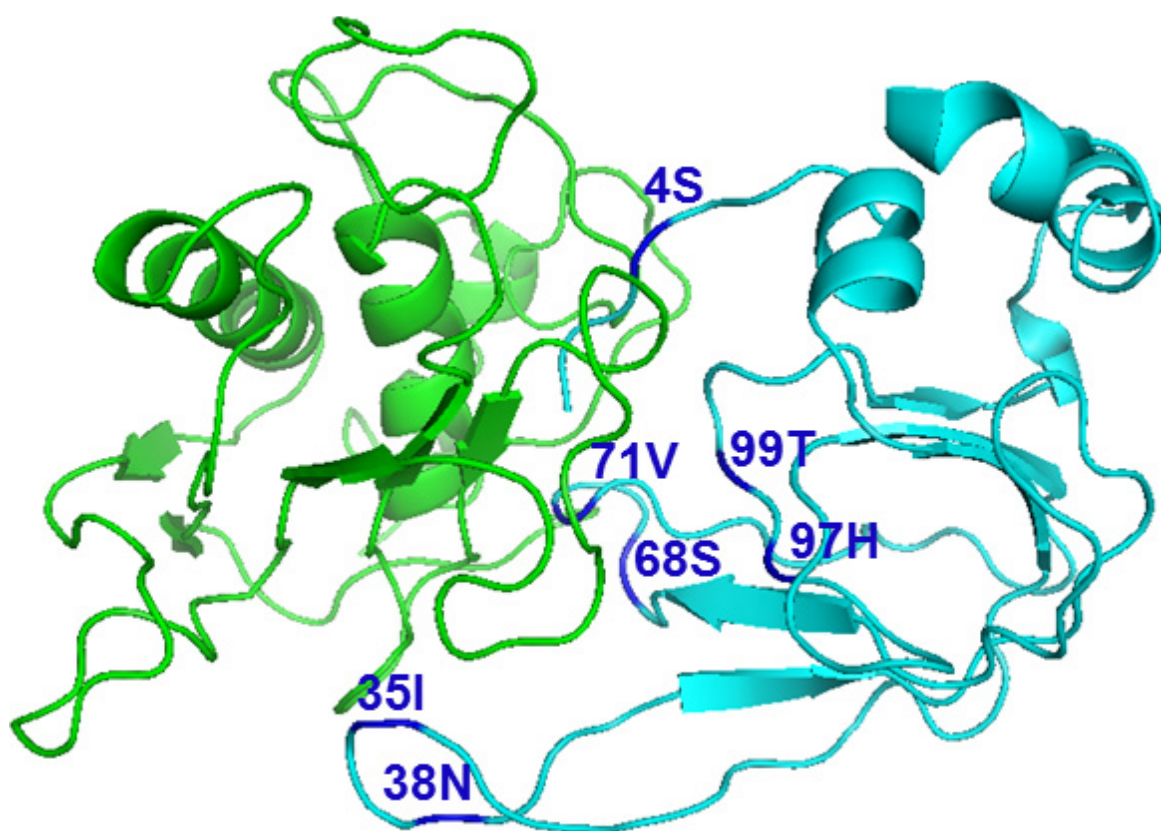
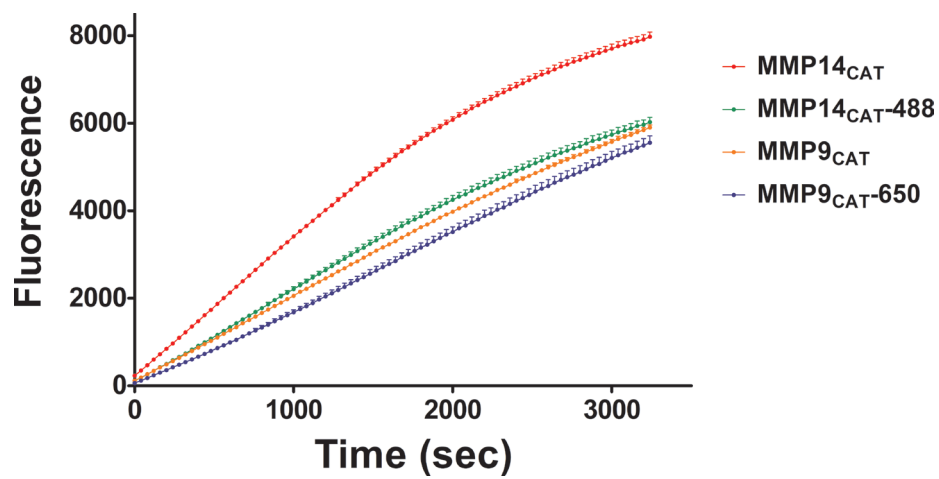


## Combinatorial engineering of N-TIMP2 variants that selectively inhibit MMP9 and MMP14 function in the cell

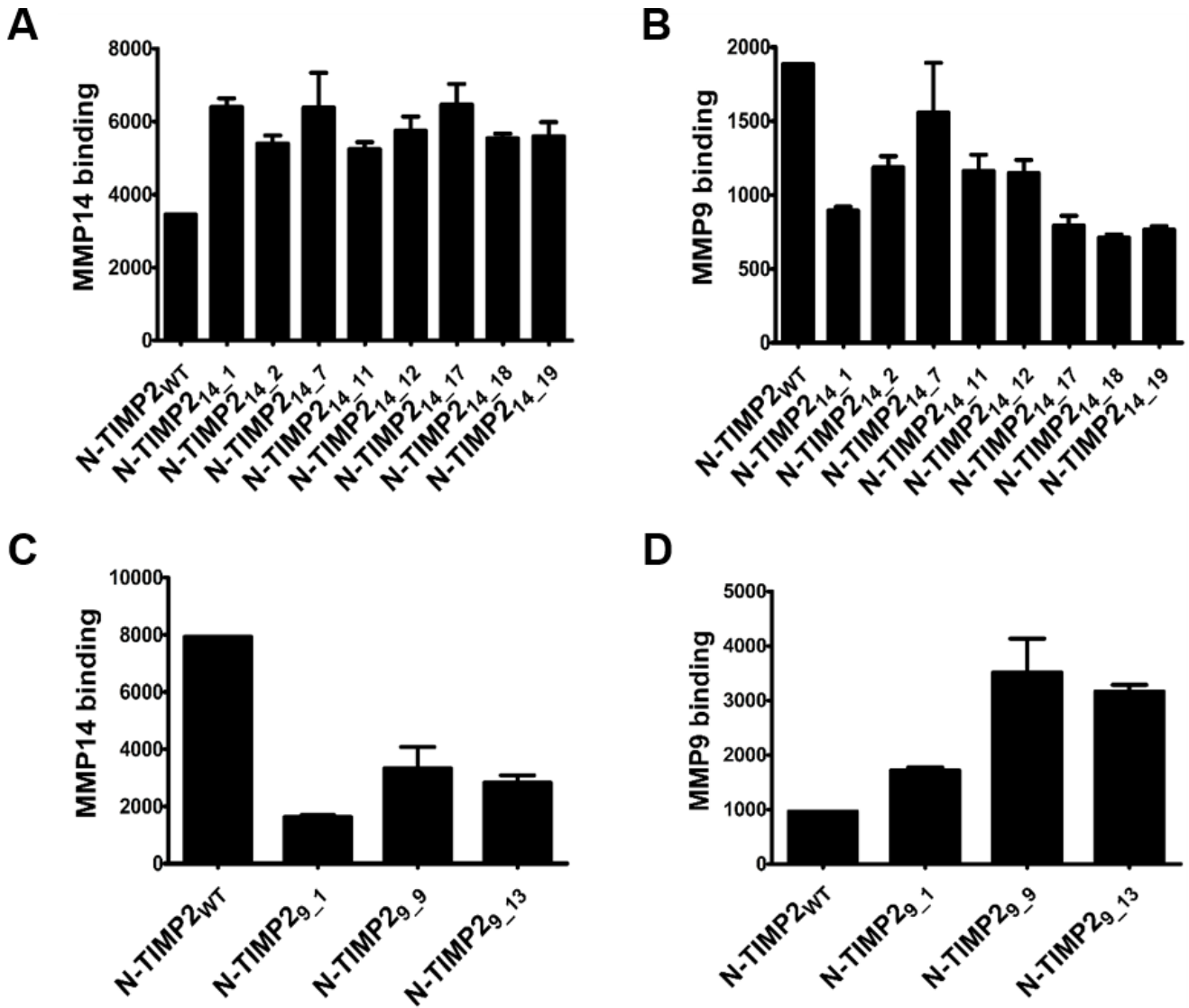
### SUPPLEMENTARY MATERIALS



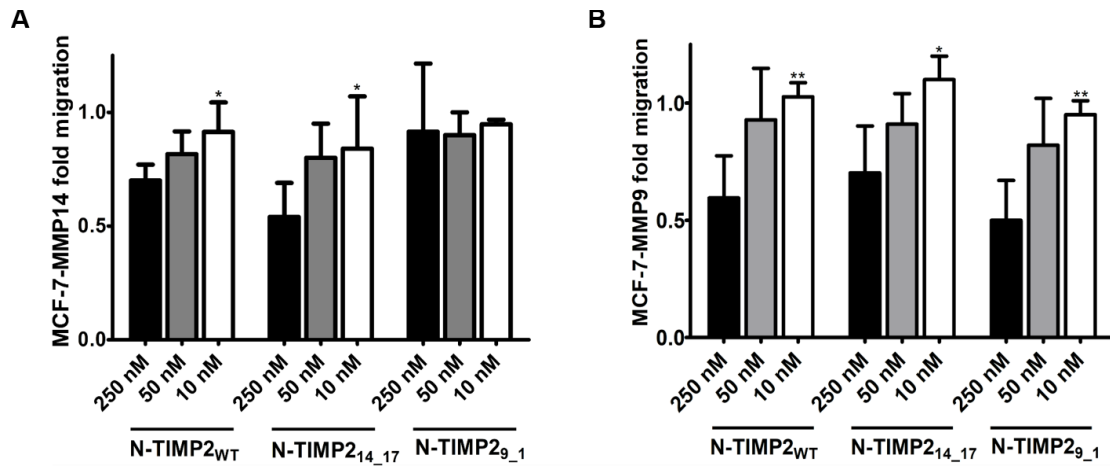
**Supplementary Figure 1: Library design.** Structure of N-TIMP2 (shown in cyan) in complex with MMP14<sub>CAT</sub> (shown in green) [adapted from PDB ID: 1BUV [58]], the positions chosen for full randomization are shown in blue.



**Supplementary Figure 2: MMP activity assay.** Cleavage of the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH<sub>2</sub>·TFA (340/30 excitation and 400/30 emission filters) at 37° C by MMP14<sub>CAT</sub> (red), DyLight-488 conjugated MMP14<sub>CAT</sub> (green), MMP9<sub>CAT</sub> (orange) and DyLight-650 conjugated MMP9<sub>CAT</sub>.



**Supplementary Figure 3: YSD binding of individual clones.** (A) YSD binding signal of MMP14-selective clones towards MMP14, as identified after the fourth round of sorting and analyzed at 100 nM of MMP14<sub>CAT</sub>-488. (B) YSD binding signal towards MMP9 of MMP14-selective clones analyzed at 100 nM of MMP9<sub>CAT</sub>-650. (C) YSD binding signal towards MMP14<sub>CAT</sub> of MMP9-binding variants identified after the fourth round of sorting analyzed at 1000 nM MMP14<sub>CAT</sub>-488. (D) YSD binding signal towards MMP9 of MMP9-binding variants identified after the fourth round of sorting analyzed at 10 nM MMP9<sub>CAT</sub>-650.



**Supplementary Figure 4: Dose-dependent selective inhibition of MMP14- and MMP9-induced migration.** (A) Calculated fold of migration, relative to the untreated control of MCF-7-MMP14 cells incubated with 250, 50 and 10 nM of the N-TIMP2<sub>WT</sub>, N-TIMP2<sub>14\_17</sub> and N-TIMP2<sub>9\_1</sub> inhibitors. The cells were counted using ImageJ software and normalized to counts of untreated cells. (B) Calculated fold of migration of MCF-7-MMP9 cells incubated with 250, 50 and 10 nM of the N-TIMP2<sub>WT</sub>, N-TIMP2<sub>14\_17</sub> and N-TIMP2<sub>9\_1</sub> inhibitors. The cells were counted as in panel (A). \* $P < 0.05$  and \*\* $P < 0.01$ , as determined in  $t$  tests comparing the fold migration at concentration of 250 nM inhibitor and 10nM inhibitor for each variant.