## **Supporting Information**

## Mechanistic studies for tri-targeted inhibition of enzymes involved in cholesterol biosynthesis by green tea polyphenols

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Figure S1. The inhibition of enzymes by ECG and EGCG. (A) The inhibition of FPPS by ECG. (B) The inhibition of FPPS by EGCG. (C) The inhibition of MVK by ECG. (D) The inhibition of MVK by EGCG. (E) The inhibition of MDD by ECG. (F) The inhibition of MDD by EGCG.



Figure S2. Fluorescence titration of FPPS by ECG, EGCG, EC and EGC.



Figure S3. Fluorescence titration of MDD by ECG, EGCG, EC and EGC.



Figure S4. Fluorescence titration of MVK by ECG, EGCG, EC and EGC.



Figure S5. Ramachandran  $\psi$ - $\Phi$  distribution plot for the final MDD homology model. Green dots represent the residues in the favorable areas (inside the green lines), yellow dots represent the residues in allowed areas (inside the orange lines), and red plus signs represent outliers.



Figure S6. Time dependence of RMSDs from production-phase MD simulations for the six energetically favorable ligand-complex systems. The blue curves stand for the RMSDs of receptor backbone atoms (C, C $\alpha$  and N), the green curves stand for the RMSDs of binding pocket residues (as far as 8 Å around ligands), and the red curves stand for the ligand RMSDs.



Figure S7. Localization properties of residue fluctuations in the complexes of the green tea polyphenols and FPPS, MVK, and MDD, as depicted through RMSFs over 50 ns MD. Blue and red curves represent the overall RMS fluctuations of receptor residues upon ECG and EGCG binding, respectively.







FPPS inhibitor: 3



FPPS inhibitor: 1



HC

FPPS inhibitor: 7

FPPS inhibitor: 11



FPPS inhibitor: 8



FPPS inhibitor: 12

FPPS inhibitor: 4



FPPS inhibitor: 13

FPPS inhibitor: 9





FPPS inhibitor: 10

FPPS inhibitor: 15

FPPS inhibitor: 16

FPPS inhibitor: 17

FPPS inhibitor: 19

FPPS inhibitor: 20

Figure S8 The 20 FPPS inhibitors for enrichment test.

FPPS inhibitor: 18



Figure S9 The 20 MVK inhibitors for enrichment test.

Out of the predicted inhibitors from 5,000 random molecules, some are experimentally proved to be FPPS or MVK inhibitors. For instance, there are some overlaps between the 5,000 random molecules and the known inhibitors. The predicted FPPS inhibitors include two known inhibitors: FPPS inhibitor 8 and 11; the predicted MVK inhibitors include three known MVK inhibitors: MVK inhibitor 2, 12, and 19. This further validates the pharmacophore models.

	Average-ECG	Average-EGCG	<b>R</b> <sup>2</sup>
FPPS	0.96	0.92	0.86
MVK	1.01	1.21	0.87
MDD	1.51	1.56	0.69

Table S1. Averages and correlation coefficients of the RMSFs of FPPS, MVK, and MDD.

Localization properties of residue fluctuations in the complexes of the polyphenols and FPPS, MVK, and MDD were studied with 50 ns MD simulations, and the root-mean-square fluctuations (RMSFs) of the residues in the complexes are depicted in **Fig. S2**. The average values of ECG and EGCG's RMSFs and the Spearman r<sup>2</sup> for correlations between ECG and EGCG curves are listed in Table S1. The MDD complex residues have higher RMSF values than other complexes, thus suggesting that MDD is more flexible. This explains why a ligand may generate an induced-fit conformational change more easily in MDD's, compared to other enzymes. Furthermore, the lower R<sup>2</sup> of MDD suggest that ECG and EGCG bind to MDD differently, while the phenols bind to FPPS and MVK in similar mode.