# Supporting Information

## Paralog specificity determines subcellular distribution, action mechanism, and anticancer activity of TRAP1 inhibitors

Hye-Kyung Park[1,§], Hanbin Jeong[1,§], Eunhwa Ko[2], Geumwoo Lee[2], Ji-Eun Lee[1], Sang Kwang Lee[2], An-Jung Lee[1], Jin Young Im[1], Sung Hu[1], Seong Heon Kim[2], Ji Hoon Lee[2], Changwook Lee[1,*], Soosung Kang[2,3,*], and Byoung Heon Kang[1,*]

[1]Department of Biological Sciences, Ulsan National Institutes of Science and Technology (UNIST), Ulsan, 44919, South Korea

[2]New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF), Daegu, 41061, South Korea

[3]College of Pharmacy and Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul, 03760, South Korea

### Content list

### Supplementary Figures

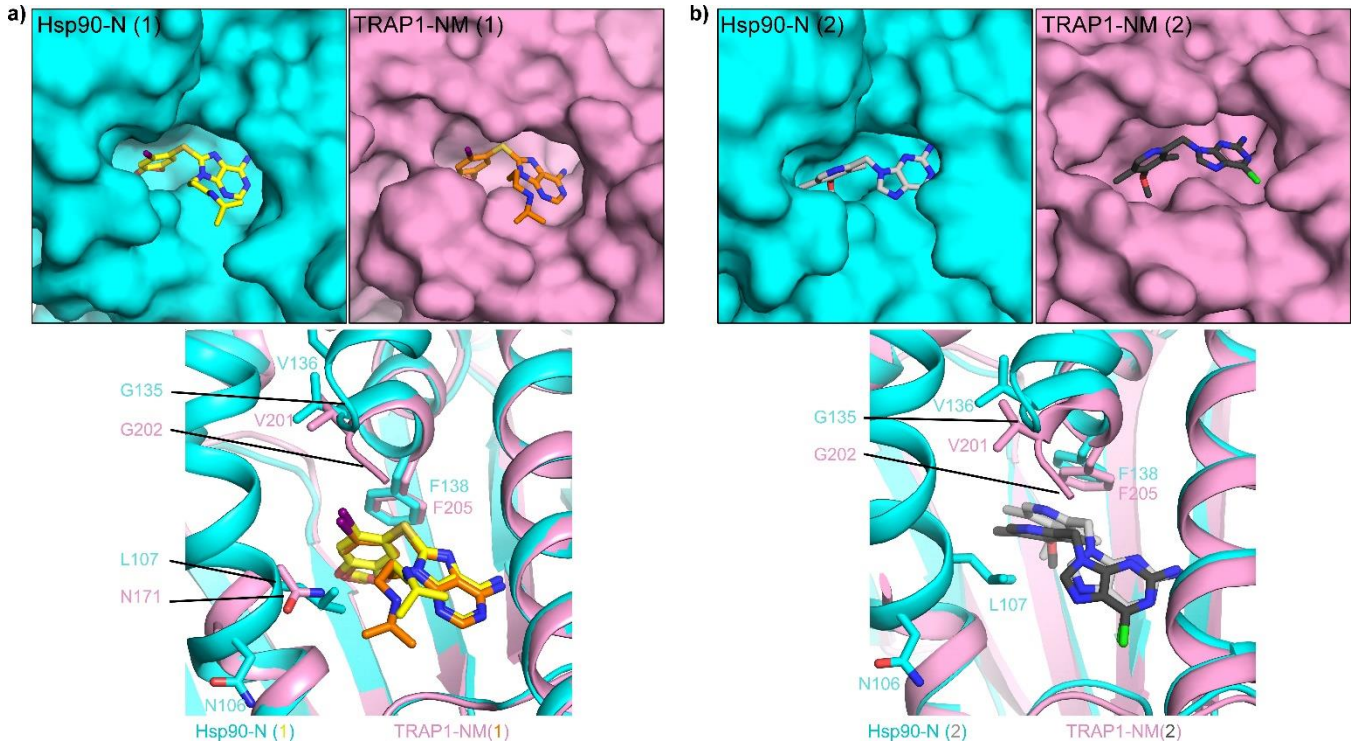


**Figure S1**. Structural comparison between Hsp90-N (PDB: 2FW2) and TRAP1-NM (PDB: 4Z1F) complexed with **1** (A) and between Hsp90-N (PDB: 3QDD) and TRAP1-NM (PDB: 4Z1G) complexed with **2** (B), respectively. Surface representation (upper) and ribbon diagram (bottom) of inhibitors bound to Hsp90 (cyan) or TRAP1 (pink) are shown.

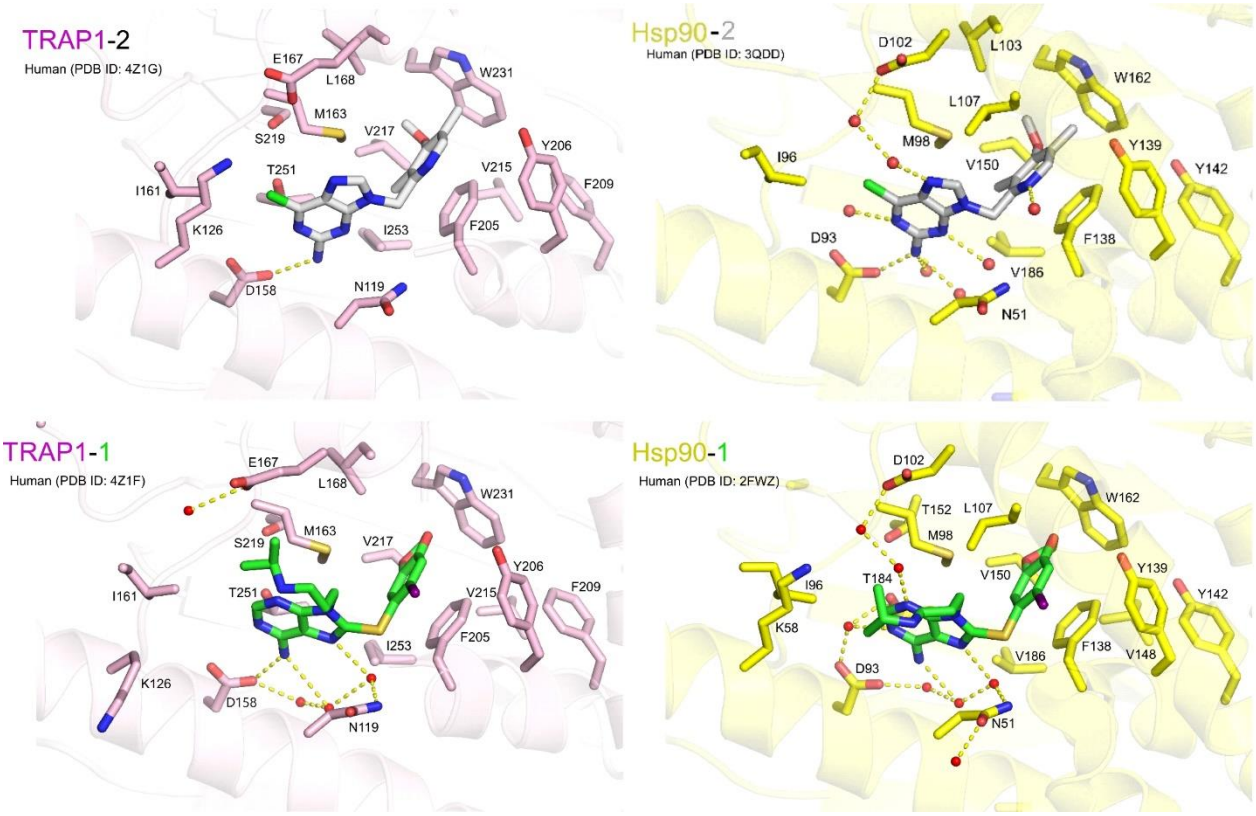


**Figure S2. Comparison of cocrystal structures of TRAP1 and Hsp90 complexed with inhibitors.** The diagram shows the comparison of the binding modes of TRAP1 (pink) and Hsp90 (yellow) with Hsp90 inhibitors, **2** (white) and **1** (green). Oxygen, nitrogen, sulfur, chlorine, and bromine are indicated by red, blue, dark yellow, green, and magenta, respectively. Yellow dotted lines and red spheres indicate intermolecular hydrogen bonds and water molecules, respectively.

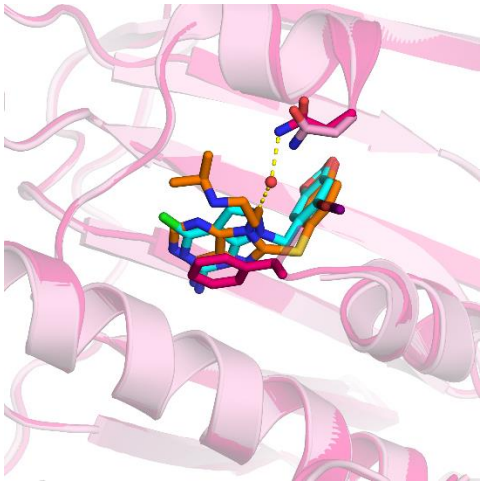

**Figure S3. Superposition of TRAP1-12b and TRAP1-1. 12b** (PDB: 5Y3N), **1** (PDB: 4Z1F), and F201 were indicated by cyan, brown, and purple, respectively. Oxygen, nitrogen, sulfur, chlorine, and bromine are indicated by red, blue, dark yellow, green, and magenta, respectively. Yellow dotted lines and red spheres indicate intermolecular hydrogen bonds and water molecules, respectively.

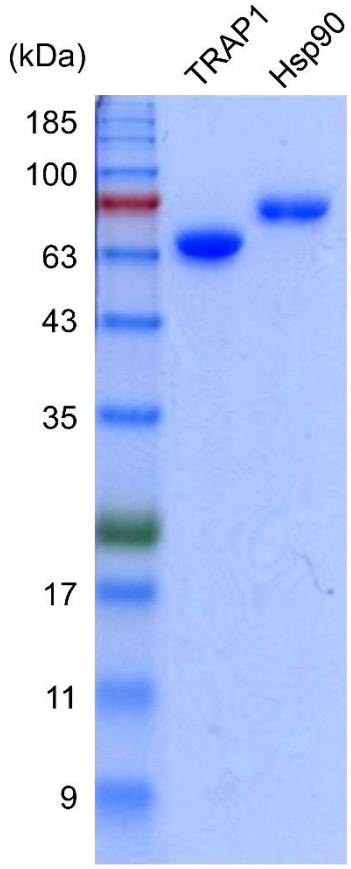


**Figure S4. Recombinant TRAP1 and Hsp90.** The purified recombinant proteins (5 μg) were analyzed by SDS-PAGE and Coomassie staining.

**Table S1.** Data collection and refinement statistics

| Ligands | **12b** (DN401) | **21** (DN320) |
|---|---|---|
| Data set: | Native | Native |
| Space group: | $P4_12_12$ | $P4_12_12$ |
| Cell parameters a, b, c (Å) | 69.4, 69.4, 252.2 | 69.5, 69.5, 253.0 |
| **Data processing** | | |
| Wavelength (Å) | 0.9796 | 0.9796 |
| Resolution (Å) | 50-2.4 | 30-2.7 |
| $R_{merge}$ (%)[a] | 7.0 (65.0)* | 8.0 (53.3) |
| I/σ | 40.0 (3.3) | 31.9 (3.5) |
| Completeness (%) | 99.1 (100.0) | 99.3 (100.0) |
| Redundancy | 6.2 (6.4) | 5.3 (5.6) |
| **Refinement statistics** | | |
| Data range (Å) | 35-2.4 | 30-2.7 |
| Reflections | 24985 | 17872 |
| Nonhydrogen atoms | 3630 | 3570 |
| Water molecules | 58 | 40 |
| Ligands | 22 | 22 |
| R.m.s. Δ bonds (Å)[b] | 0.004 | 0.007 |
| R.m.s. Δ angles (°)[b] | 0.782 | 1.139 |
| R-factor (%)[c] | 20.8 | 21.8 |
| $R_{free}$ (%)[c, d] | 25.7 | 27.1 |
| Ramanchandran plot, residues in | | |
| Most favored (%) | 92.8 | 93.9 |
| Additional allowed (%) | 6.3 | 5.1 |
| Generously allowed (%) | 1.0 | 1.0 |
| Disallowed (%) | 0 | 0 |

*Highest resolution shell is shown in parenthesis.
[a]$R_{merge} = 100 \times \sum_h \sum_i | I_i(h) - \langle I(h) \rangle | / \sum_h \langle I(h) \rangle$, where $I_i(h)$ is the $i$th measurement and $\langle I(h) \rangle$ is the weighted mean of all measurement of $I(h)$ for Miller indices h.
[b]Root-mean-squared deviation (r.m.s. Δ) from target geometries.
[c]R-factor $= 100 \times \sum |F_P - F_{P(calc)}| / \sum F_P$.
[d]$R_{free}$ was calculated with 5% of the data.