

## **Supplemental data**

### **Crystallographic and mutational analysis of the CD40-CD154 complex and its implications for receptor activation**

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**Table S1. Crystallographic Statistics**

|   | CD40-CD154          | CD40-CD154(S154W)   |
|---|---------------------|---------------------|
| <b>Data collection</b>                              |                     |                     |
| Space group   | P6 <sub>5</sub>     | P6 <sub>5</sub>     |
| Cell dimensions                                     |                     |                     |
| <i>a</i> , <i>b</i> , <i>c</i> (Å)                  | 133.2, 133.2, 211.2 | 133.9, 133.9, 214.2 |
| Resolution (Å)                                      | 50.0 – 3.5          | 50.0 – 5.0          |
| <i>R</i> <sub>sym</sub>                             | 0.102 (0.351) *     | 0.177 (0.286) *     |
| <i>I</i> / <i>σI</i>                                | 8.9 (2.2) *         | 6.2 (3.2) *         |
| Completeness (%)                                    | 93.7 (82.3) *       | 95.0(90.3) *        |
| Redundancy  | 2.6                 | 3.2                 |
| Processing program                                  | Mosflm/SCALA        | HKL2000             |
| Beam line   | ID29, ESRF          | BL41XU, SPring-8    |
| <b>Search probe for molecular replacement</b>       |                     |                     |
|   | 1ALY                |                     |
| <b>Refinement program</b>                           |                     |                     |
|   | CNS 1.3             |                     |
| Resolution (Å)                                      | 50.0 – 3.5          |                     |
| No. reflections (work/test)                         | 23648 / 1209        |                     |
| <i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub> | 0.245 / 0.298       |                     |
| No. atoms   |                     |                     |
| Protein   | 9900                |                     |
| Water   | 0                   |                     |
| Average B factors                                   | 69.4                |                     |
| R.m.s deviations                                    |                     |                     |
| Bond lengths (Å)                                    | 0.010               |                     |
| Bond Angles (°)                                     | 1.4                 |                     |
| NCS rmsd (Å)  | 0.133               |                     |
| Ramachandran Plot (%)                               |                     |                     |
| Favored   | 69.4                |                     |
| Allowed   | 28.3                |                     |
| Generously allowed                                  | 2.3                 |                     |
| Disallowed  | 0.0                 |                     |

\* Numbers in the parentheses are calculated with data in the highest resolution shell.

CD40 EP-----PTA 25

**CRD1**

|       |  |           |
|-------|--|-----------|
| CD40  | CREKQYLIN---SQCCSLCQPGQKLVSDCT-EFTETECLP       | 61        |
| TNFR1 | CPOGKYIHPQNNNSICCTKCHKGTLYLNDPCPGPGQDTDCRE     | 83        |
| DR5   | <u>PQORAAPOQKRSSPSEGLCPPGHHISEDGR-----DCIS</u> | <u>96</u> |

A module                      B module

**CRD2**

|       |   |            |
|-------|---|------------|
| CD40  | CGE-SEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICT      | 104        |
| TNFR1 | CES-GSFTASENHLRHCLSCSKCRKEMGQVEISSCTVDRDRTVCG     | 126        |
| DR5   | <u>CKYGQDYSTHWNDLLFCLRCRCD--GEVELSPCTTTRNTVCQ</u> | <u>138</u> |

A module                      B module

**CRD3**

|       |   |            |
|-------|---|------------|
| CD40  | CEEG--WHCTSEACESCVLHRS-CSPGFGVKQIATGVSDTICEP        | 145        |
| TNFR1 | CRKNQYRHYWSENLFQCFNCSL-CLNGT-VHLSCQEKQNTVCT-        | 167        |
| DR5   | <u>CEEG--TFREEDSPEMCRKCRGTGCPGMVKVGDCTPWSDIECVH</u> | <u>180</u> |

A module                      B module

CD40 CPVGGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGPQD 190

**Figure S1.** Sequence alignment of CD40 with other TNF receptor family proteins of known structure. Cysteines linked by disulfide bridges are connected by solid lines. The cys111-cys116 disulfide bridge is unique in CD40 and linked in red. Residues included in the crystallized protein but omitted in the final coordinate file are in grey; they showed very weak electron density presumably due to high structural flexibility.