



**Supplementary figure S1.** Comparison of the HER2 epitope recognized by 2Rs15d and those reported for other described HER2 binders (shown in a grey cartoon representation), and a detailed view of HER2-2Rs15d interactions. The color code is as in Fig. 1A. **(A)** Superposition of HER2-2Rs15d with HER2-DARPin 9\_26 (PDB ID: 4HRM, (1)), HER2-DARPin 9\_29 (PDB ID: 4HRL, (1)), HER2-DARPin G3 (PDB ID: 4HRN, (1)), HER2-Affibody Z<sub>HER2:342</sub> (PDB ID: 3MWZ, (2)). **(B)** Superposition of HER2-2Rs15d with HER2-pertuzumab (PDB ID: 1S78, (3)), HER2-trastuzumab (PDB ID: 1N8Z, (4)), HER2-scFv chA21 (PDB ID: 3H3B, (5)), HER2-Fab 37 (PDB ID: 3N85, (6)). **(C)** The left panel shows a figure very similar to Fig. 1A, with the difference that

the sdAb CDRs are colored (CDR1, blue; CDR2, green, CDR3, orange). The right panel shows a close up of the interaction between 2Rs15d (red cartoon representation) and HER2 domain I (tan cartoon representation). The other HER2 domains are not shown for reasons of clarity. The CDRs of 2Rs15d are colored as in the left panel. The six lysines (grey) and the four cysteines (yellow) of 2Rs15d are shown in stick representation. Note that all cysteines are involved in disulfide bonds and that none of the lysines are part of the 2Rs15d paratope. **(D)** Stereo views of the HER2-2Rs15d interactions. The interacting residues are shown in stick representation and are indicated by the colored labels. Water molecules are shown as red spheres and the dashed lines indicate hydrogen bonds or salt bridges (distance values between 2.68 Å and 4.23 Å; see also Supplementary Table S2).

#### References:

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