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Supplemental Information

Structure of the Discoidin Domain Receptor 1 Extracellular Region Bound to an Inhibitory Fab Fragment Reveals Features Important for Signaling

Federico Carafoli, Marie Cathrin Mayer, Kazushige Shiraishi, Mira Anguelova Pecheva, Lai Yi Chan, Ruodan Nan, Birgit Leitinger, and Erhard Hohenester

Inventory of Supplemental Information

Figure S1

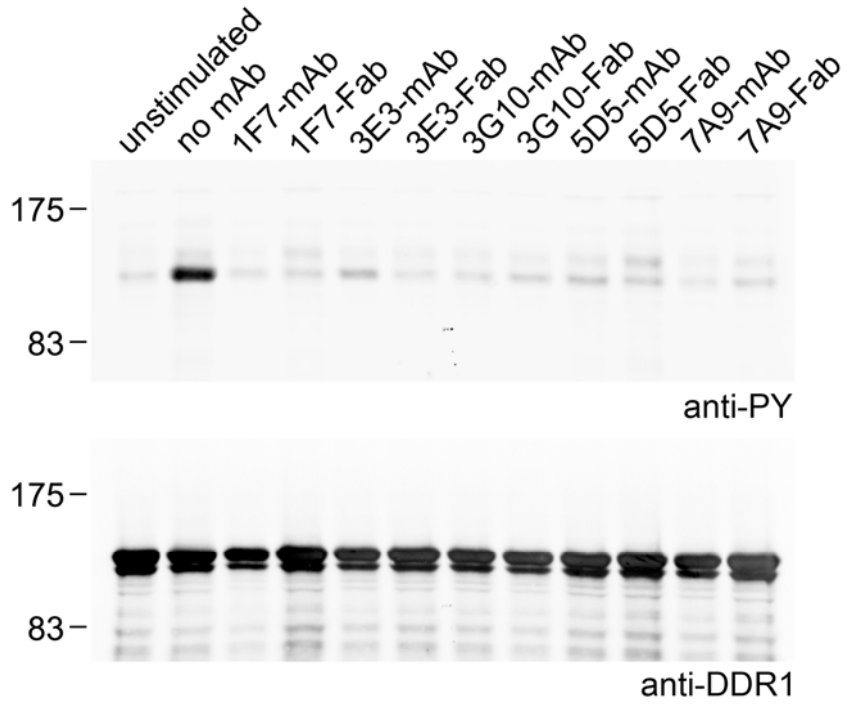

unstimulated
no mAb
1F7-mAb
1F7-Fab
3E3-mAb
3E3-Fab
3G10-mAb
3G10-Fab
5D5-mAb
5D5-Fab
7A9-mAb
7A9-Fab
175
83
anti-PY
175
83
anti-DDR1

Figure S2

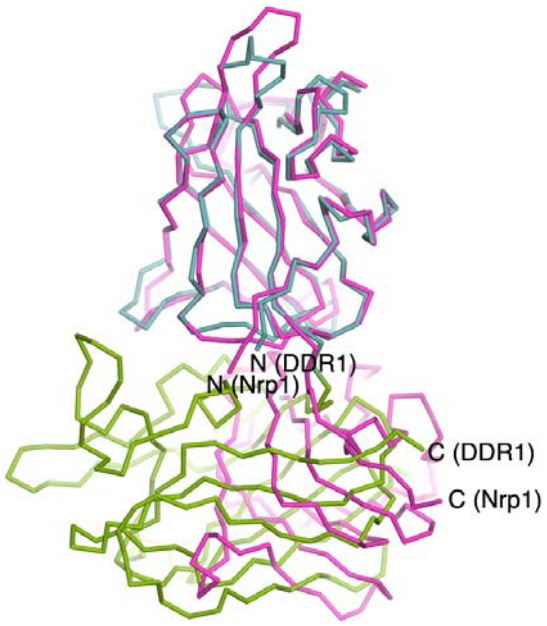


Figure S3

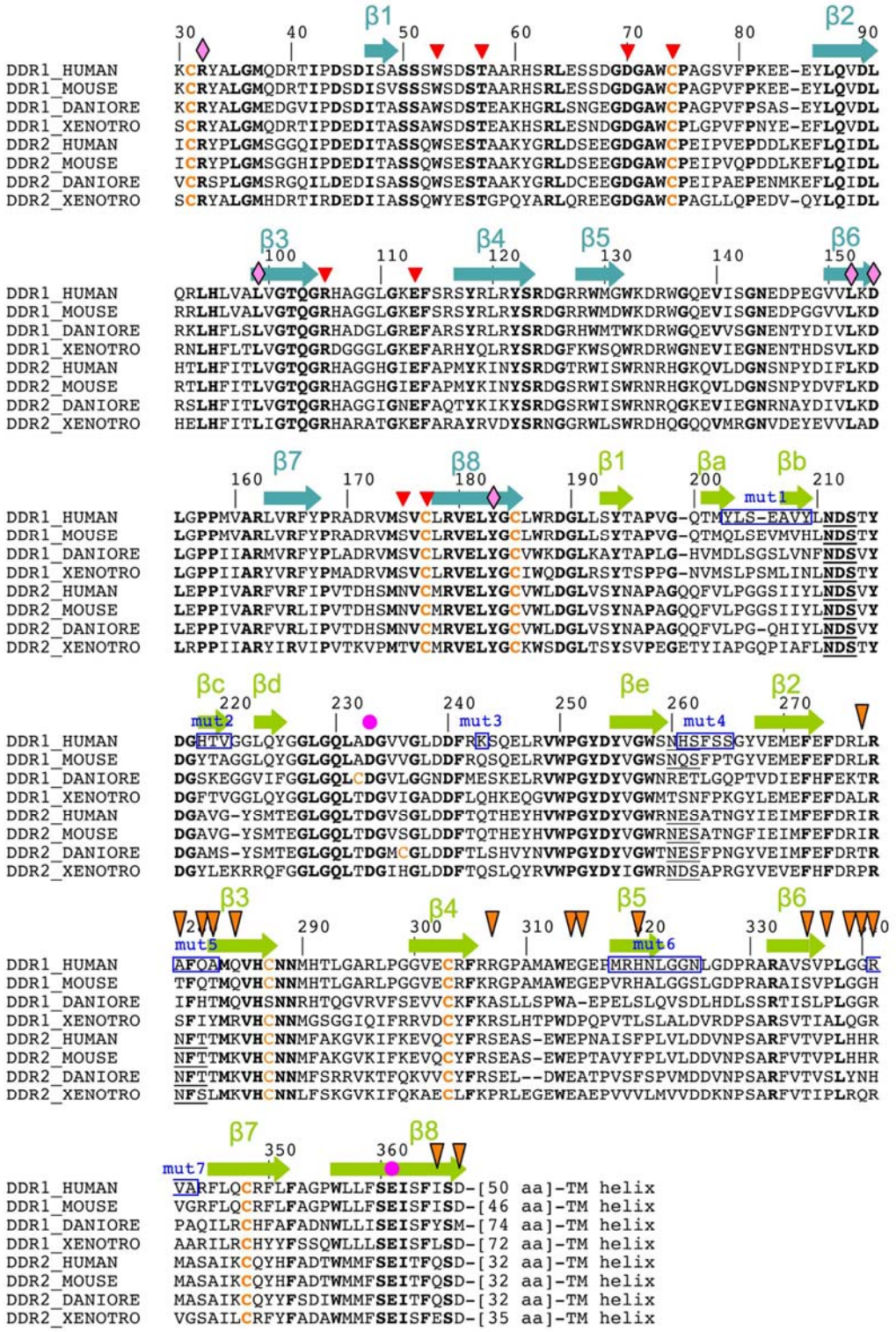
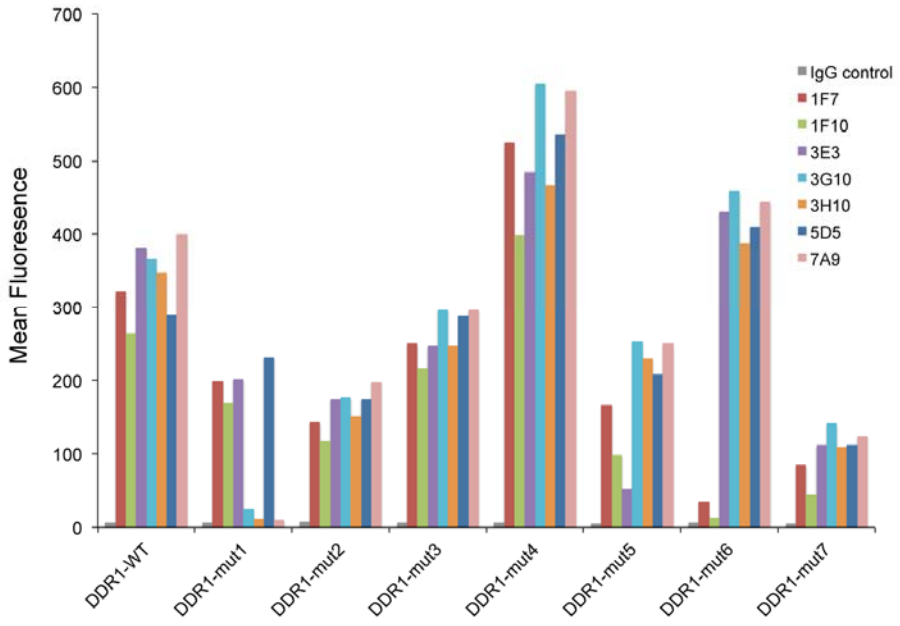

β1
β2
β3
β4
β5
β6
β7
β8
βa
βb
βc
βd
βe
mut1
mut2
mut3
mut4
mut5
mut6
mut7
DDR1_HUMAN
DDR1_MOUSE
DDR1_DANIORE
DDR1_XENOTRO
DDR2_HUMAN
DDR2_MOUSE
DDR2_DANIORE
DDR2_XENOTRO
[50 aa]-TM helix
[46 aa]-TM helix
[74 aa]-TM helix
[72 aa]-TM helix
[32 aa]-TM helix
[32 aa]-TM helix
[32 aa]-TM helix
[35 aa]-TM helix

Figure S4


Mean Fluoresence
700
600
500
400
300
200
100
0
IgG control
1F7
1F10
3E3
3G10
3H10
5D5
7A9
DDR1-WT
DDR1-mut1
DDR1-mut2
DDR1-mut3
DDR1-mut4
DDR1-mut5
DDR1-mut6
DDR1-mut7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Selected Fab fragments block collagen-induced DDR1 phosphorylation. DDR1b was transiently expressed in HEK293 cells. The cells were stimulated for 90 min at 37° C with 10 μg/ml collagen I in the presence or absence of the indicated anti-DDR1 mAbs or Fab fragments (at 10 μg/ml). Aliquots of cell lysates were analysed by SDS-PAGE and Western blotting. The blots were probed with anti-phosphotyrosine (anti-PY) mAb 4G10 (upper blot) and re-probed with anti-DDR1 Abs (lower blot). The experiment was performed three times with very similar results.

Figure S2, related to Figure 3. Superposition of DDR1 (cyan, DS domain; green DS-like domain) and the DS domain pair of neuropilin-1 (Nrp1, magenta) (Vander Kooi et al., 2007). The DDR1 DS domain was fitted to the first DS domain (b1) of Nrp1. The DDR1 DS-like domain and the second DS domain (b2) of Nrp1 are related by a ~60° rotation about a vertical axis.

Figure S3, related to Figure 3. Alignment of selected DDR1 and DDR2 sequences (*Homo sapiens* DDR1, Q08345; *Mus musculus* DDR1, Q03146; *Danio rerio* DDR1, XP_001345829; *Xenopus tropicalis* DDR1, XP_002939505; *Homo sapiens* DDR2, Q16832; *Mus musculus* DDR2, Q62371; *Danio rerio* DDR2, XP_684261; *Xenopus tropicalis* DDR2, XP_002933824). The numbers above the alignment refer to the human DDR1 sequence. Conserved residues are in bold and cysteines are in orange. Predicted *N*-linked glycosylation sites are underlined. The secondary structure elements of the DDR1 structure are indicated above the alignment. Red inverted triangles indicate key collagen-binding residues in DDR2 (Carafoli et al., 2009). Magenta filled circles indicate calcium ligands in DDR1. Pink

diamonds indicate residues contributing to the conserved surface patch in the DS domain. Orange inverted triangles indicate residues involved in 3E3 Fab binding (>10 $Å^2$ reduction in solvent accessibility upon Fab binding). The seven linear and non-conservative human-to-mouse substitutions in the DS-like domain are boxed in blue and labelled mut1-7.

Figure S4, related to Figure 6. Mean fluorescence values of the flow cytometry data shown in **Figure 6A**. DDR1b wild-type or the indicated DDR1 mutants were transiently expressed in HEK293 cells. The cells were stained on ice with 10 μg/ml of the indicated anti-DDR1 mAbs or mouse IgG1 isotype control Ab followed by FITC-conjugated goat-anti mouse IgG and analysis by flow cytometry.