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Reconstructing the TIR side of the Myddosome: a paradigm for TIR-TIR interactions?

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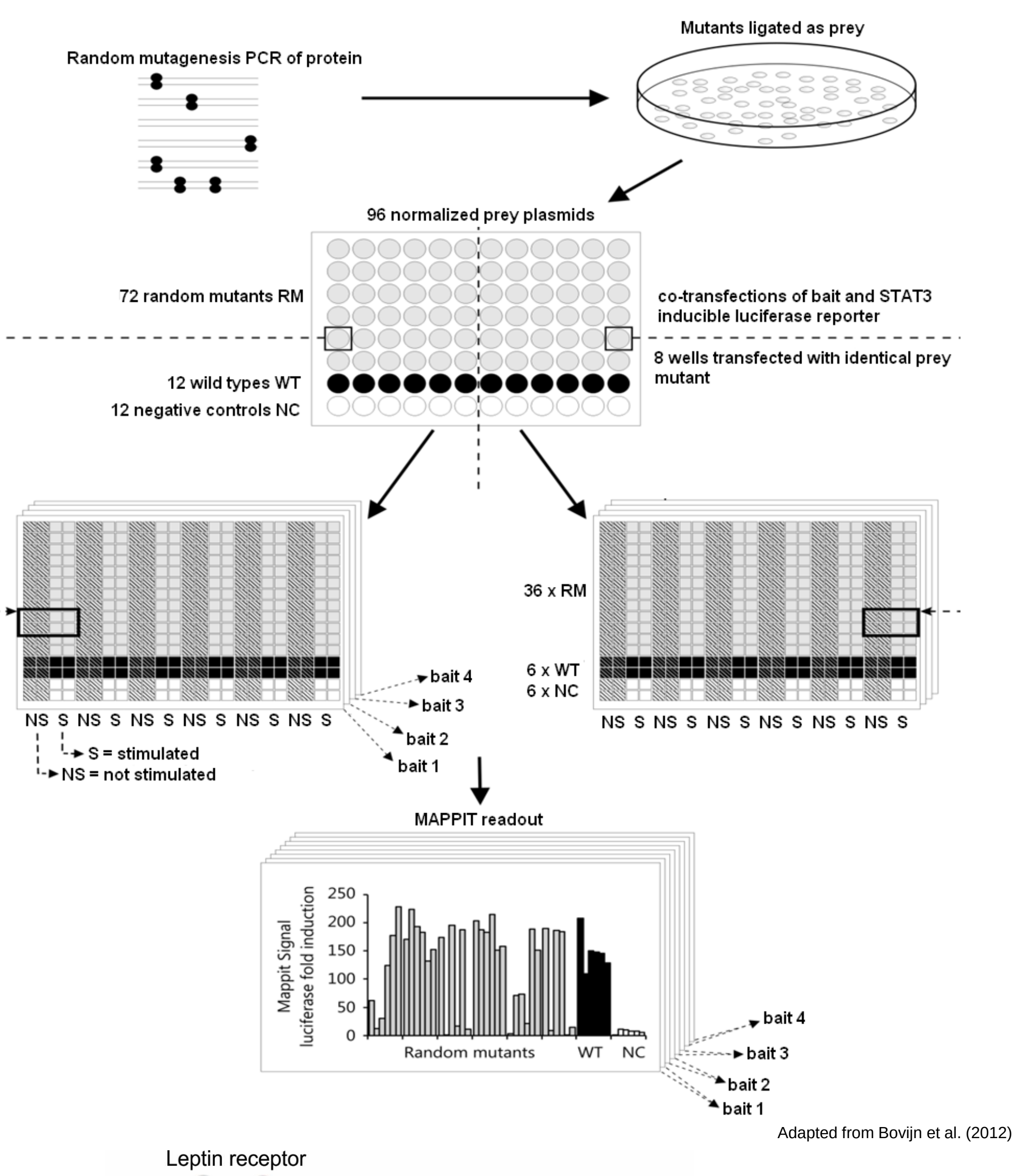
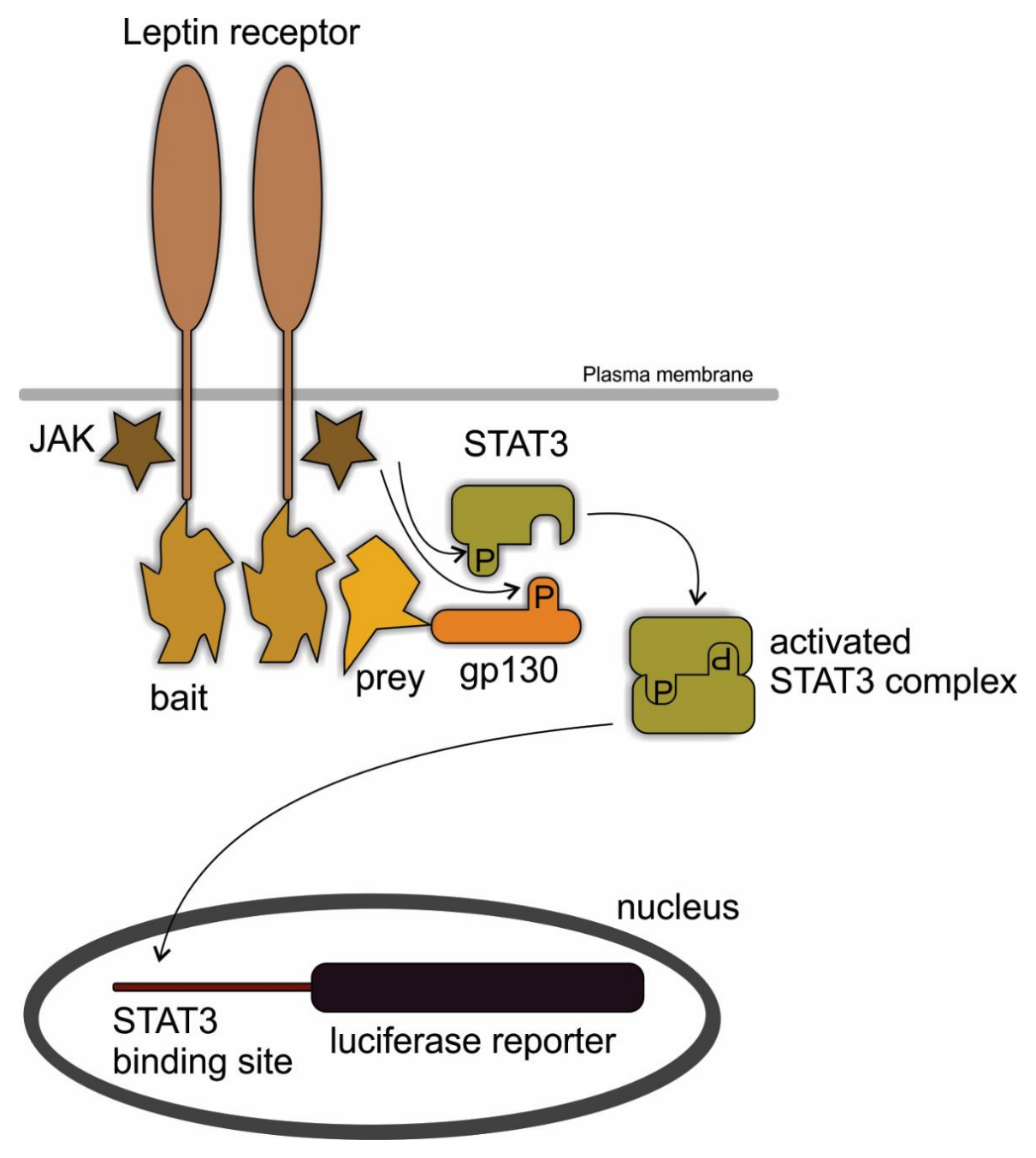
By combining the mammalian two-hybrid system MAPPIT and saturation mutagenesis, we complement and extend crystallographic and NMR data, and reveal how TIR domains interact. Our approach fully delineates the interaction sites on the MyD88 TIR domain for homo-oligomerization and for interaction with Mal and TLR4. Interactions between three sites drive MyD88 homo-oligomerization. The BB-loop interacts with the αE-helix, explaining how BB-loop mimetics inhibit MyD88 signaling. The αC'-helix interacts symmetrically. The MyD88 TIR domains thus assemble into a left-handed helix, compatible with the Myddosome death domain crystal structure. Our assembly explains regulation of MyD88 by Mal, phosphorylation, and oncogenic mutations. These findings provide a paradigm for the interaction of mammalian TIR domains.

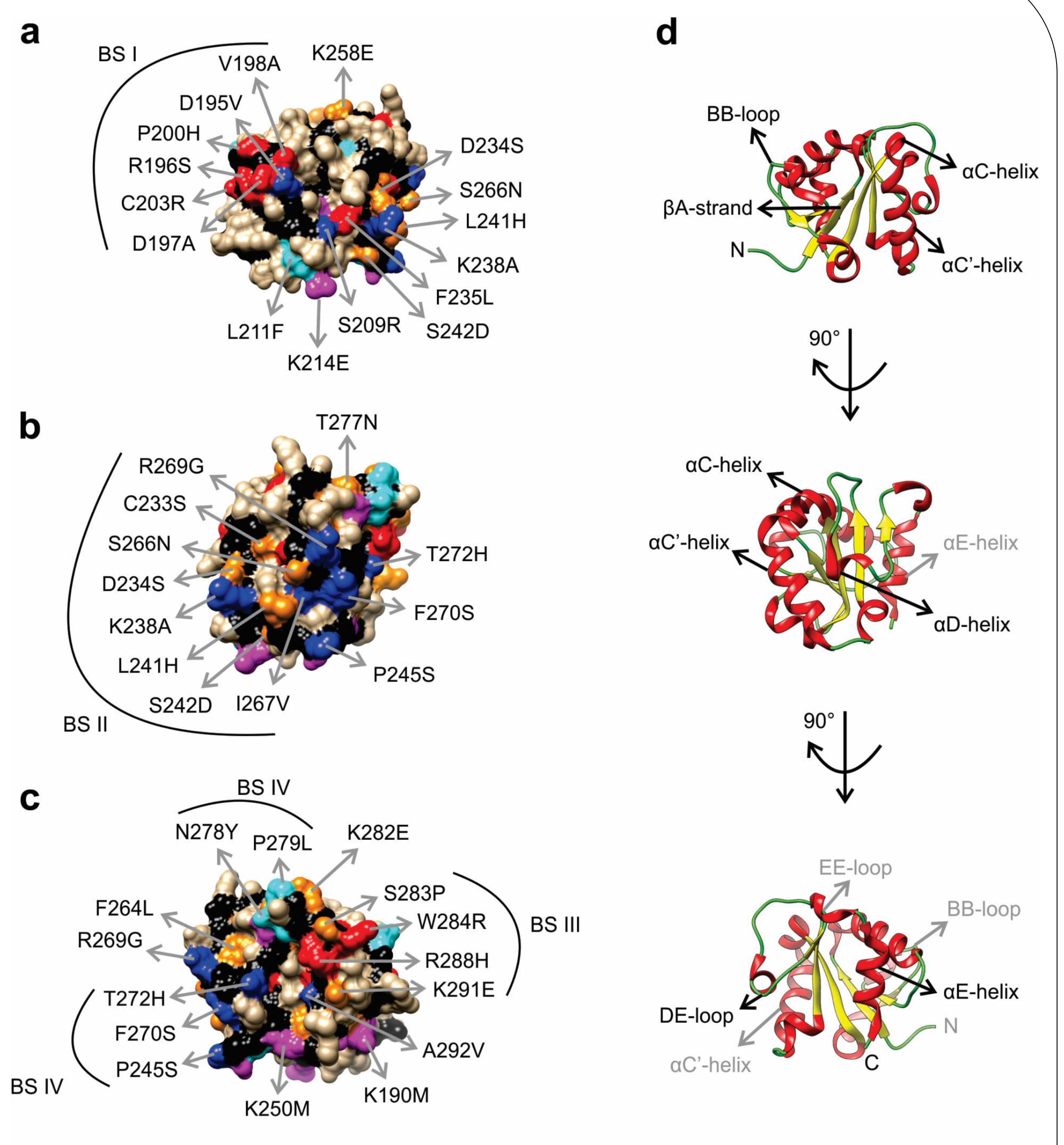
Random mutagenesis and MAPPIT



Adapted from Bovijn et al. (2012)



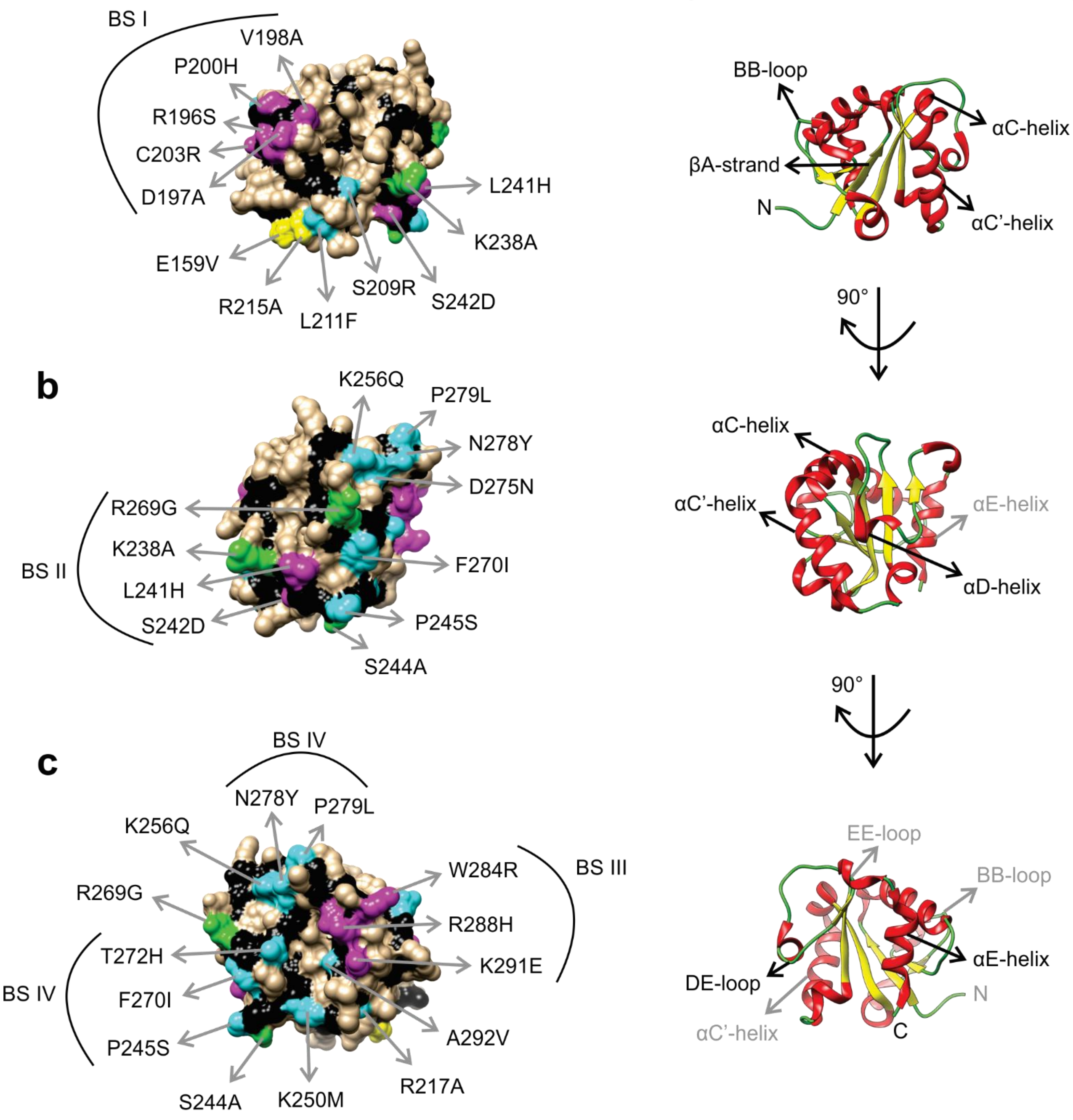
MAPPIT is based on the JAK-STAT signaling pathway of class I cytokine receptors. Upon ligand binding, the leptin receptor complex is reorganized, leading to recruitment and cross-activation of associated Janus kinases (JAKs). The bait protein is fused to the mutated intracellular part of the leptin receptor, which is deficient for the recruitment of Signal Transducer and Activator of Transcription 3 (STAT3). The prey protein is tethered to a fragment of the gp130 receptor chain which harbours several STAT3 recruitment sites. Upon binding of prey and bait, STAT3 is phosphorylated and forms an activated STAT3 complex. This complex migrates to the nucleus, resulting in the induction of a STAT3-responsive reporter gene.



	Mal bait	MyD88 bait
cyan	< 60%	≥ 75%
blue	< 30%	≥ 75%
magenta	< 60%	≥ 60% and < 75%
orange	< 60%	< 60%
red	< 30%	< 30%

(a) The first binding site "I" (BS I) includes the residues of the BB-loop. Mutations at these positions strongly affect the interaction with MAL, MyD88 and TLR4. (b) A second binding site "II" (BS II) is found around the C-terminal half of the αC'-helix. Mutations at the center of this binding site affect the MyD88 TIR-MyD88 dimerization assay. (c) The third binding site "III" (BS III) is situated at the N-terminal half of the αE-helix. Similarly to BS I, mutations at this site strongly affect all testes interaction assays. A fourth site "IV" (BS IV) consists mostly of residues of the DE-loop and EE-loop. Mutations in this site more specifically affect the interaction with MAL and TLR4ic. Non-mutated residues are uncolored, and the backbone is indicated in black.

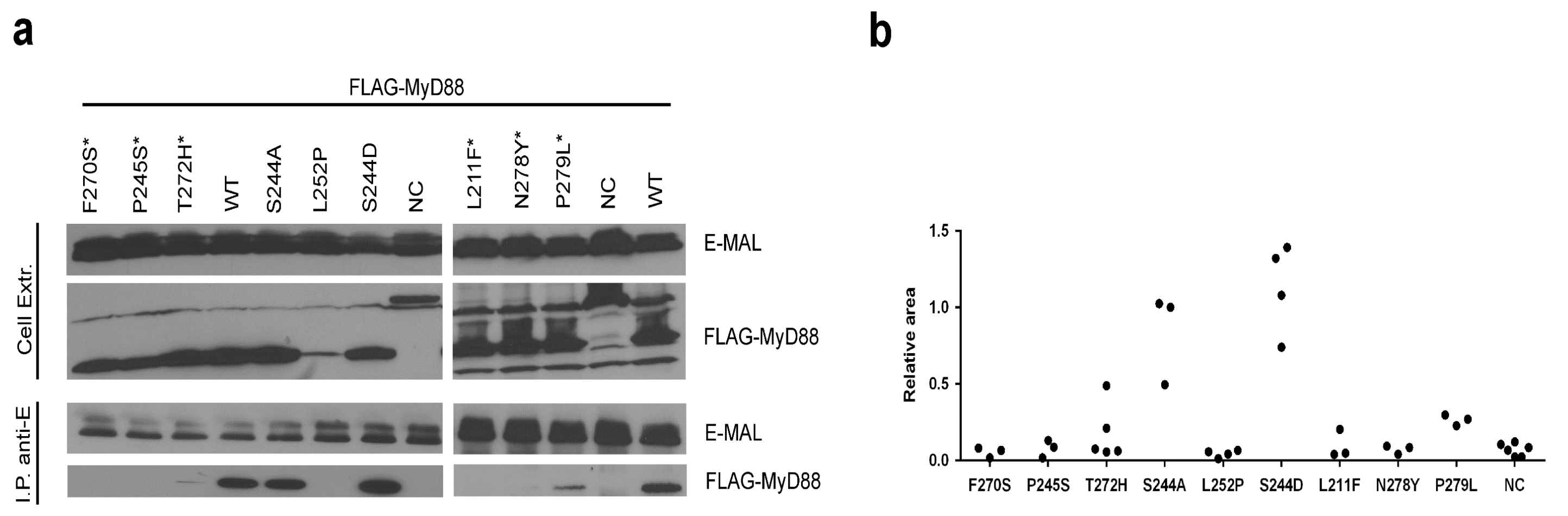
NF-κB activation



	Mal bait	MyD88 bait	NF-κB activation
yellow	≥ 60%	≥ 60%	< 70%
cyan	< 60%	≥ 60%	≥ 70%
magenta	< 60%	< 60%	< 60%
green	< 60%	≥ 60%	< 70%

(a, b, and c) Mutations in BS I, BS III, and in the center of BS II strongly reduce the NF-κB activation, corroborating the effect of these mutations on MyD88-MyD88 interaction. (c) Mutations in BS IV have no appreciable effect on the NF-κB reporter activation, in line with a specific role of this site in MyD88-MAL interaction. Non-mutated residues are uncolored, and the backbone is indicated in black.

MyD88-Mal Co-IP

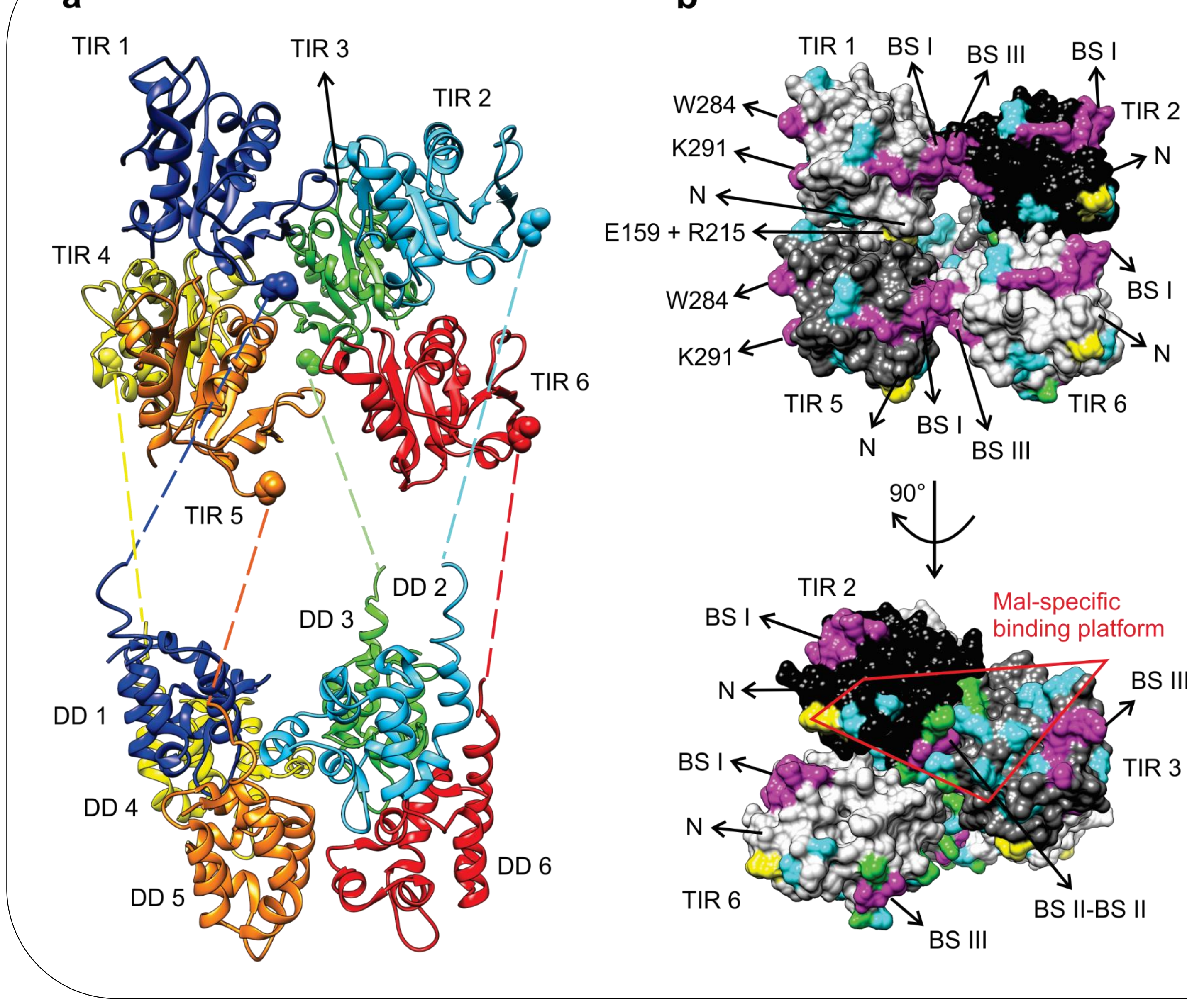


BS IV Mutations in MyD88 Disrupt Mal-Binding in a Co-Immunoprecipitation Assay.
(a) We co-transfected FLAG-tagged wild-type or mutant MyD88 with E-tagged Mal. FLAG-tagged SVT was transfected as a negative control. All mutations in BS IV, indicated by *, abolish the interaction with Mal (all p-values < 0.01), while the S244A and S244D mutations do not disrupt MAL-binding. The L252P mutation (p < 0.0001) also abolishes binding with MAL, but this could be due to lower expression of the mutant. (b) Densitometric analysis of the effect of MyD88 mutants on MAL-binding. Data are represented as relative area from three up to five independent experiments.

Molecular dynamics

S244D and L252P mutations affect the dynamics and position of the αC'-helix in MyD88 TIR, which may enhance interactions via BS II. Molecular dynamics simulations are performed on the S244D (cyan) and L252P (yellow) MyD88 TIR mutants, and on the MyD88 TIR wild-type (black). The αC'-helix is located in the front.

Hexameric MyD88 TIR assembly



(a) The left-handed helical MyD88 TIR model (top) closely resembles the organization of the MyD88 death domains in the Myddosome crystal structure (bottom). (b) Alternating site I-III and site II-II interactions lead to the helical MyD88 TIR model. A large Mal-specific binding site is exposed, containing BS IV and residues S209 and L211.