Supplementary information

Paired yeast one-hybrid assays to detect DNA-binding cooperativity and antagonism across transcription factors

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Supplementary Figures

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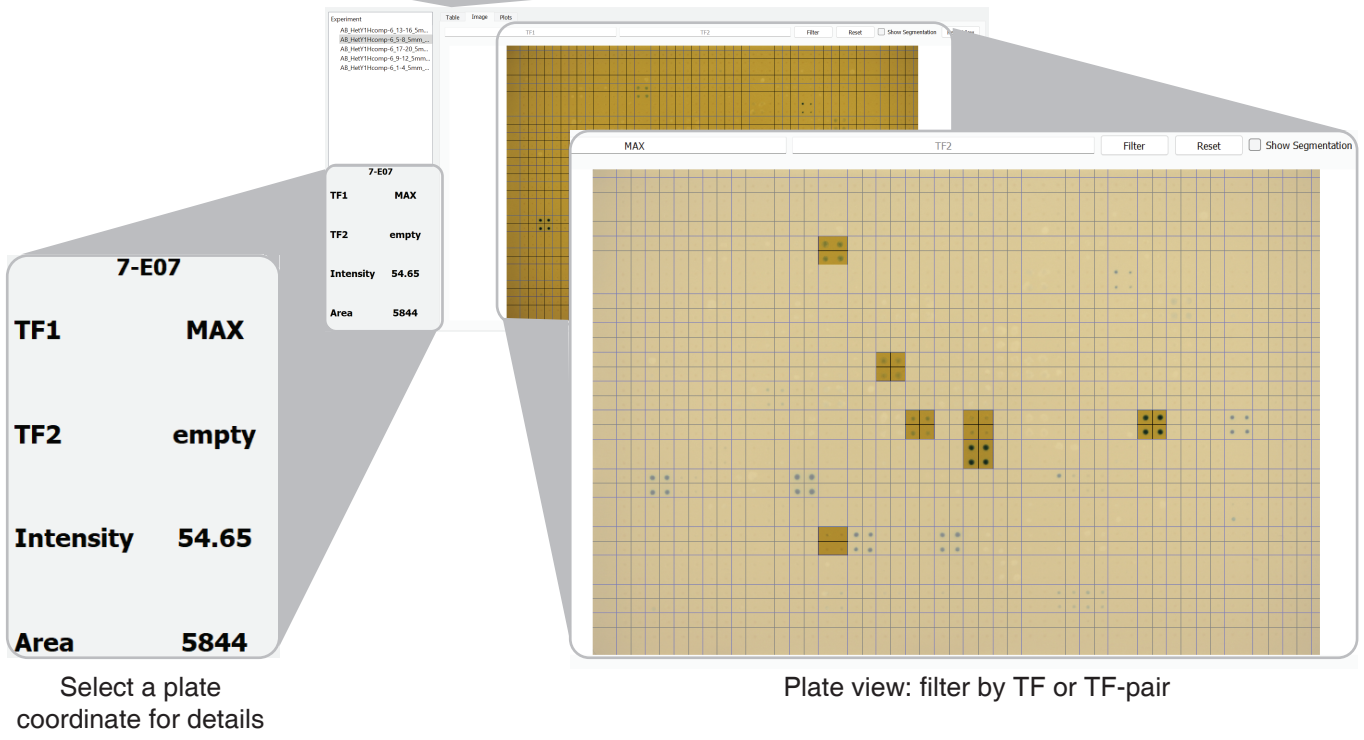**Supplementary Figure 9:** Predicted structures of STAT1-STAT3 dimers
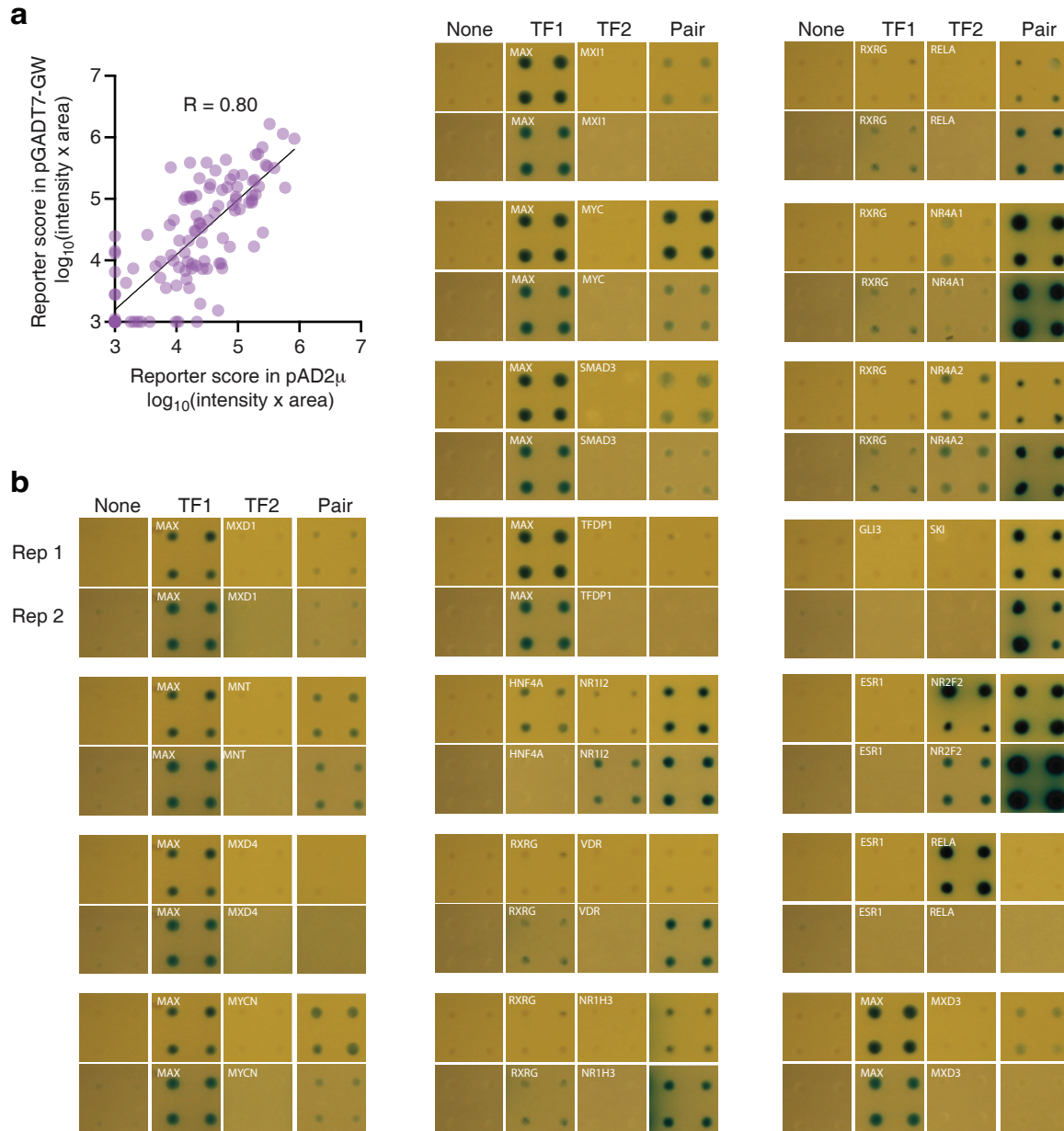
Supplementary Figure 1. Reproducibility of pY1H assays. (**a**) Correlation between reporter scores (intensity x area) for single-TFs (n = 120) cloned into the pGADT7-GW and the pAD2μ vectors and transformed into yeast. Correlation by Pearson Correlation Coefficient. (**b**) Cooperative and antagonistic interactions from two pY1H screens using the 2-AD design conducted on different days for the *CCL15* promoter. Source data are provided as a Source Data file.
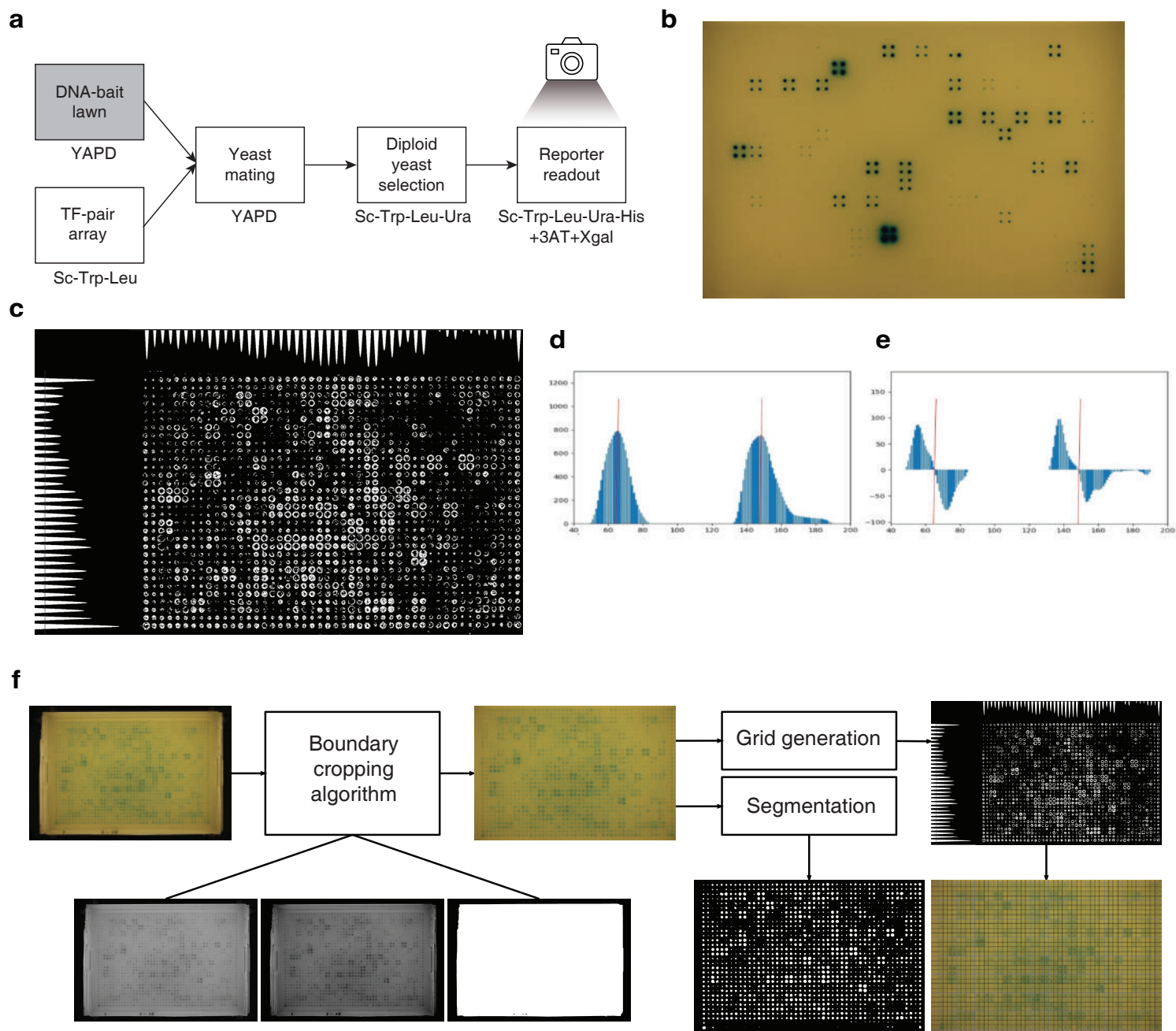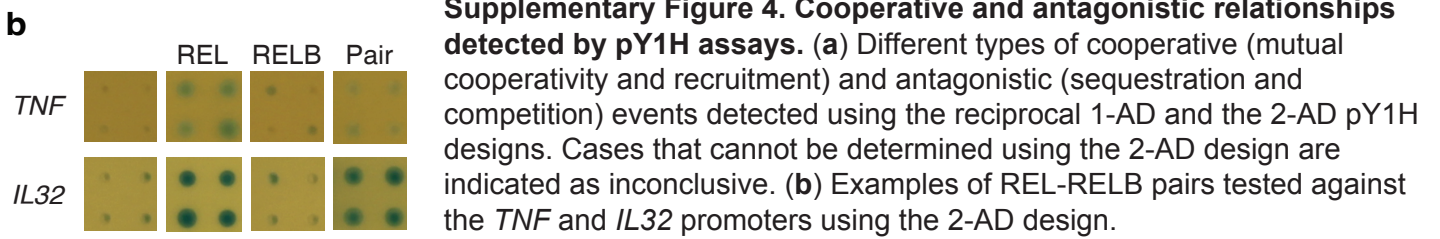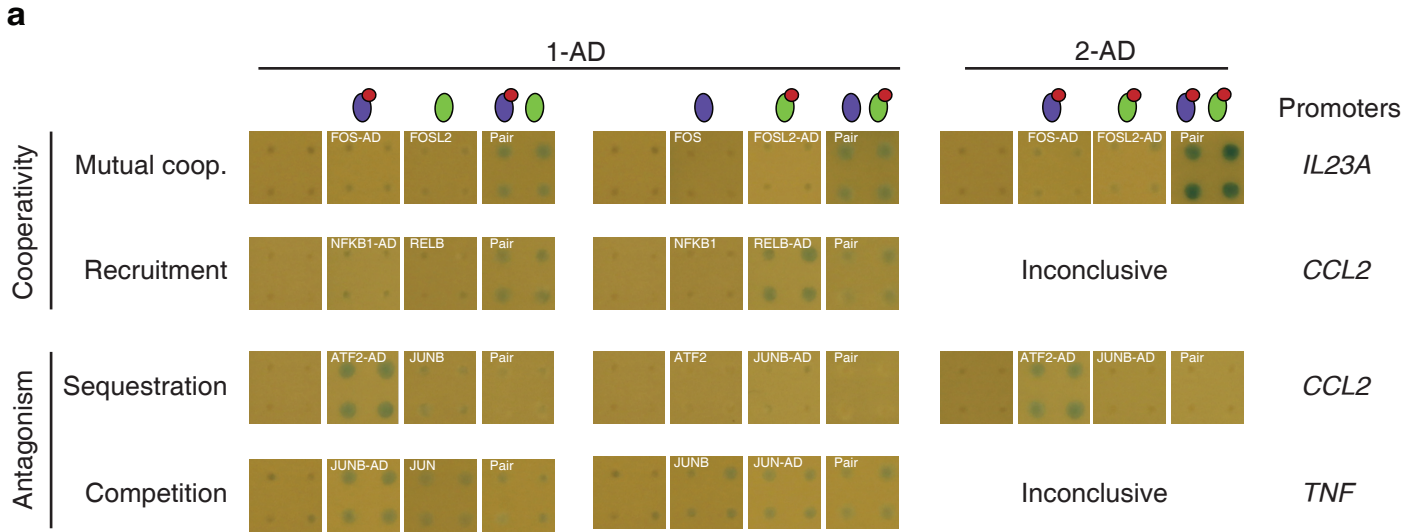
Supplementary Figure 2. pY1H experimental and analysis pipeline. (**a**) Experimental pipeline for pY1H assays. A lawn of the yeast DNA-bait is mated with the TF-pair array in a YAPD plate for 1 day. Diploid yeast are selected in Sc-Trp-Leu-Ura plates for 2 days and then transfered to readout plates containing 3AT and X-gal. Pictures are taken at days 2, 3, 4 and 7. (**b**) Example of a pY1H readout plate. (**c**) Vertical and horizontal histogram projection of a sub-optimal segmentation mask. Each peak corresponds to the center of the colony. (**d**) Plot of a 160-pixel region of histogram projection. (**e**) Plot of the first-order derivative of the region of the plot in d. Each "zero-crossing" event corresponds to the histogram's peak or the colony's center. (**f**) Pipeline of the image analysis process. First, the boundary cropping algorithm selects the region of interest. Then the grid generation algorithm performs histogram projection analysis to generate a grid based on the TF-pair array coordinates. Finally, the segmentation model generates a segmentation map for the colonies and determine colony area and intensity of blue color.
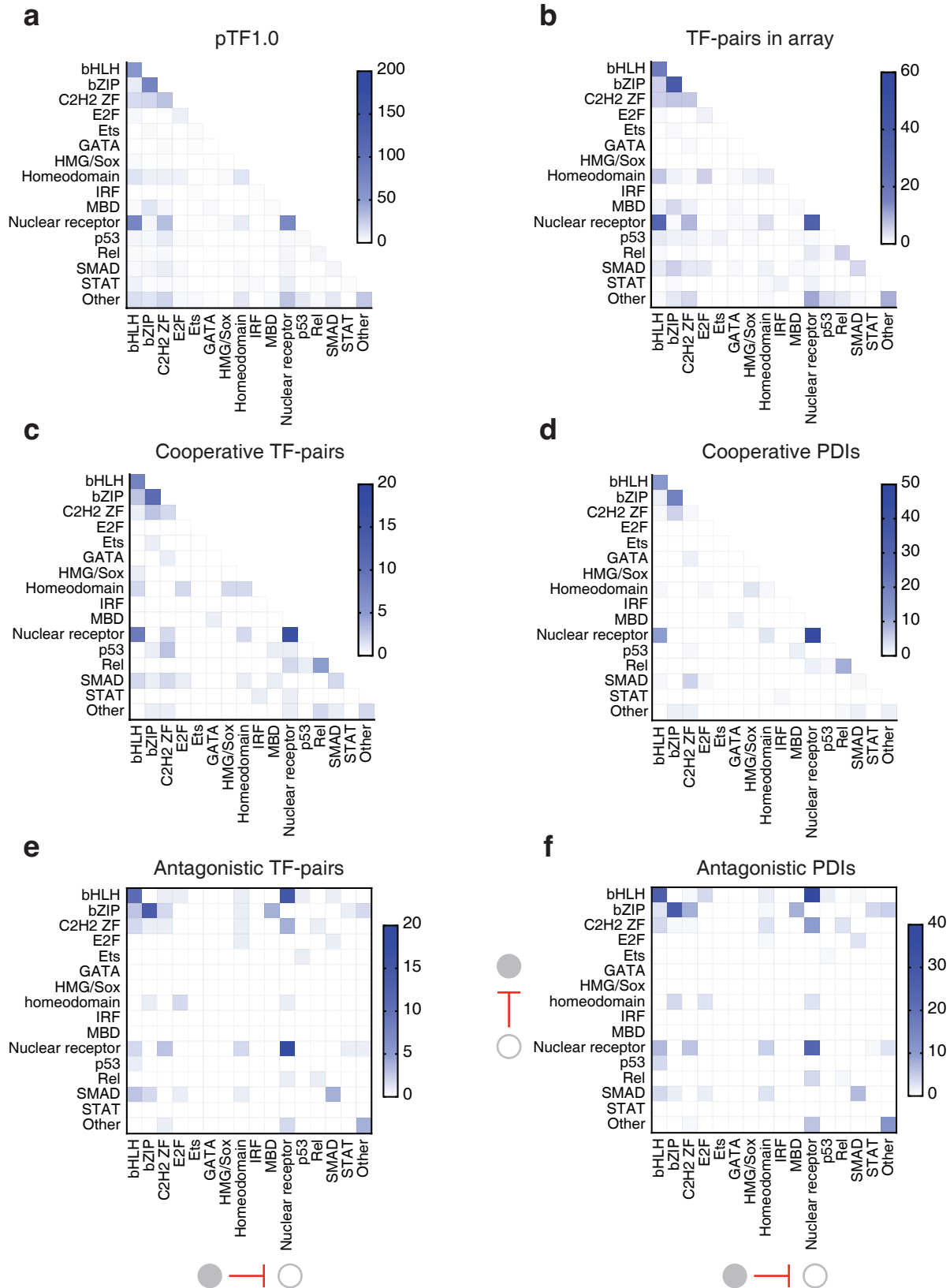
Table view: assembles TF-pairs and single-TF control strains

	TF1	TF2	Cooperative Index	Antagonism Index-1	Antagonism Index-2	Intensity	Area	Image	TF1-Empty Intensity	TF1-Empty Area	TF1-Empty Image	TF2-Empty Intensity	TF2-Empty Area	TF2-Empty Image
35	MAX	MXD3	-280798	280805	-38569.4	14.56	2649		54.65	5844		0	0	
36	MAX	MXI1	-303601	303608	-15766.3	10.74	1468		54.65	5844		0	0	
38	MAX	MYC	-37420.9	37427.9	-281947	47.82	5896		54.65	5844		0	0	

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TF1	**MAX**
TF2	**empty**
Intensity	**54.65**
Area	**5844**

Select a plate coordinate for details

Plate view: filter by TF or TF-pair

Supplementary Figure 3. Features of DISHA. The Plate view displays plate images derived from pY1H assays and allows to filter by singe-TF or TF-pair. Users can also select an array coordinate to identify the TF-pair, reporter intensity measured as blue color, and area of colonies. The Table view displays images of TF-pair colonies alongside single-TF colonies allowing side-by-side comparisons, together with reporter intensity and colony area. In addition, the table presents the cooperative and antagonistic indices calculated for each TF-pair.

Supplementary Figure 4. Cooperative and antagonistic relationships detected by pY1H assays. (**a**) Different types of cooperative (mutual cooperativity and recruitment) and antagonistic (sequestration and competition) events detected using the reciprocal 1-AD and the 2-AD pY1H designs. Cases that cannot be determined using the 2-AD design are indicated as inconclusive. (**b**) Examples of REL-RELB pairs tested against the *TNF* and *IL32* promoters using the 2-AD design.
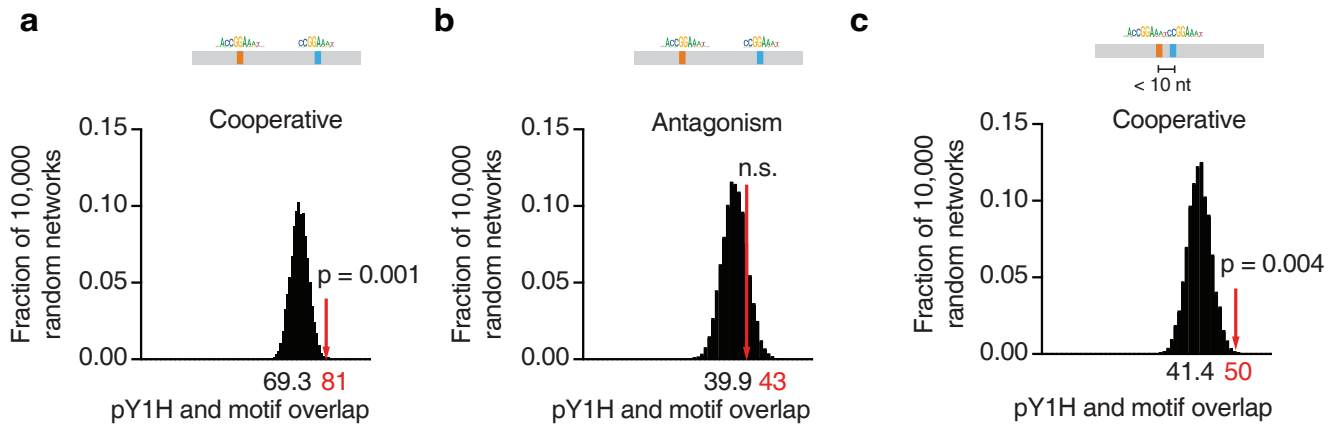
Supplementary Figure 5. TF family representation. (**a, b**) Number of TF-pairs for each TF family combination for the 868 TF-pairs in pTF1.0 (**a**) and the 297 sequence-confirmed TF-pairs in the array (**b**) (as in Fig. 3b,c). (**c, d**) Number of cooperative TF-pairs (**c**) and PDIs (**d**) identified in a pY1H assay against 18 cytokine promoters. (**e, f**) Number of antagonistic TF-pairs (**e**) and PDIs (**f**) identified in a pY1H assay against 18 cytokine promoters. Columns indicate the family of the antagonistic TF and rows indicate the family of the antagonized TF. Source data are provided as a Source Data file.

a

Cooperative

b
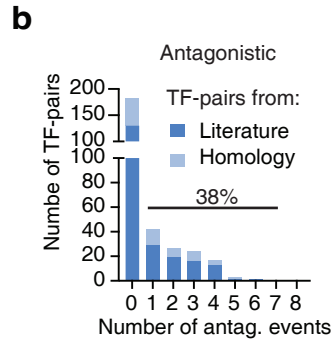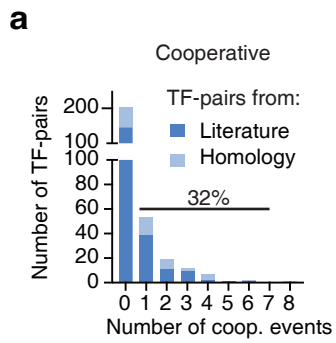
Antagonistic

Supplementary Figure 6. Cooperative and antagonistic events from pY1H assays. Number of TF-pairs involved in different numbers of cooperative (**a**) and antagonistic (**b**) events for TF-pairs derived from the literature or predicted based on homology. The percentage of TF-pairs with at least one cooperative or antagonistic event is indicated. Source data are provided as a Source Data file.

a Cooperative — Fraction of 10,000 random networks — p = 0.001 — pY1H and motif overlap — 69.3 81

b Antagonism — n.s. — Fraction of 10,000 random networks — pY1H and motif overlap — 39.9 43

c Cooperative — < 10 nt — Fraction of 10,000 random networks — p = 0.004 — pY1H and motif overlap — 41.4 50

Supplementary Figure 7. Overlap between pY1H data and motif predictions. (**a, b**) Overlap between pY1H cooperative (**a**) and antagonistic (**b**) events with the presence of motifs for both single-TFs in the corresponding promoter sequences. The histograms show the distribution of the overlap with each randomized pY1H network, where the pY1H network was randomized 10,000 times by edge-switching (each edge is a TF-pair and a cytokine promoter). The numbers under the histograms indicate the average overlap in 10,000 randomized networks, while the red arrows indicate the observed overlap with the actual pY1H-derived network. Two-tailed statistical significance was calculated from Z-score values assuming normal distribution for overlap with the randomized networks. (**c**) Overlap between pY1H cooperative events with the presence of motifs for both single-TFs within 10 bp in the corresponding promoter sequences. Source data are provided as a Source Data file.
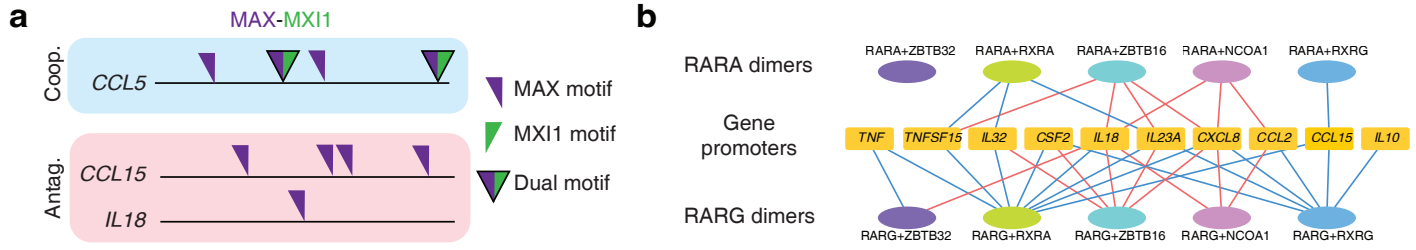
Supplementary Figure 8. DNA binding specificity for TF-pairs. (**a**) Distinct and overlapping motifs for MAX and MXI1 in the indicated cytokine gene promoters. (**b**) Cooperative and antagonistic events for TF-pairs involving paralogs RARA and RARG. Source data are provided as a Source Data file.
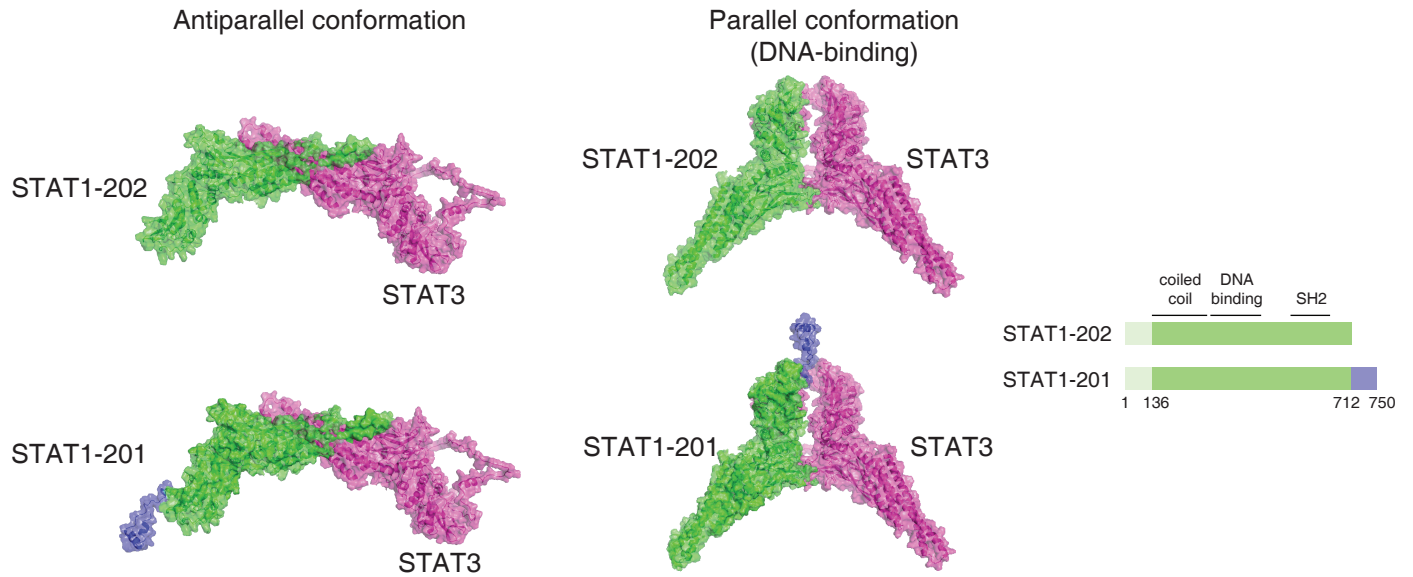
Supplementary Figure 9. Predicted structures of STAT1-STAT3 dimers. Structures of STAT1 isoforms and reference STAT3 isoform predicted using Alphafold 2 were arranged in the antiparallel conformation (unbound to DNA) or parallel conformation (adopted for DNA binding). Amino acids 136-712 of STAT1 are colored in green, the additional sequence (713-750) of STAT1-201 is colored in blue, and STAT3 is colored in magenta.