

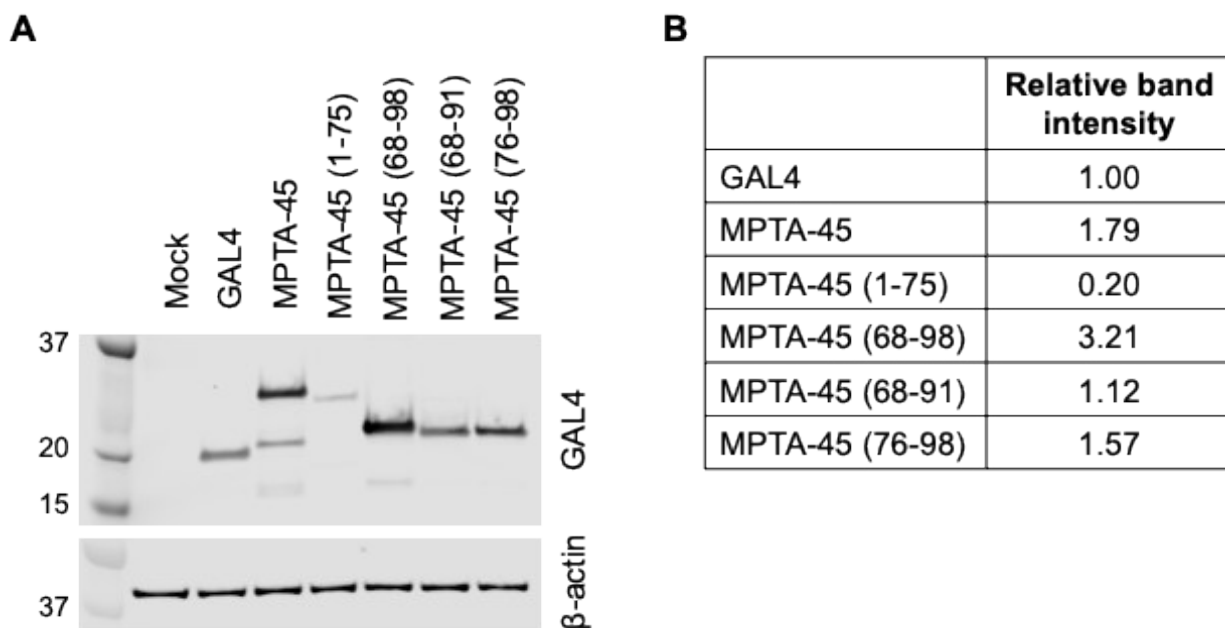
## **Supplementary Information**

### **Identification of microproteins with transactivation activity by polyalanine motif selection**

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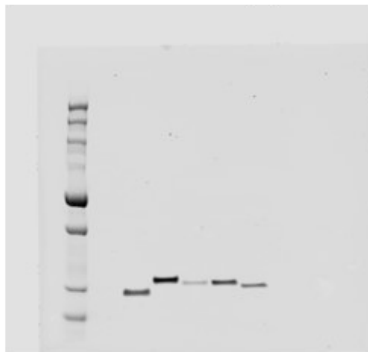
**Figure S1.** HEK293T cells were transfected with MPTA-45 variants, and Western blot was performed with GAL4-antibody and  $\beta$ -actin-antibody to detect protein expression (A) Western blot (B) The relative protein band intensities for MPTA-variants in respective lanes were normalized to  $\beta$ -actin and quantified with ImageJ. The experiment was done once.

MPTA-10 AGAVLAAAAAAEGGRLCPGLCLPSSAAVPW  
MPTA-17 MPGTAAAVAAAAAATATAATAA**GPGPGWGLESLQWG**  
MPTA-45 MGFFSEDTEARLPDAHPIREGALPLPSCRGMASRRGGRERSAETREGP  
YPGERQLPTSGRASGEAL**PVAAAAAAVVPGVFVPPVSLPWP**AAFSRL

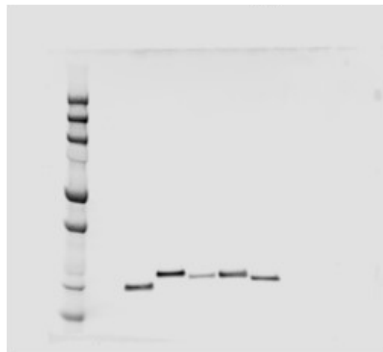
**Figure S2.** Microprotein sequences of MPTA-10, 17 and 45 with transactivation sequence identified in MPTA-17 and MPTA-45 in bold.

## Supplementary Data Western Blots

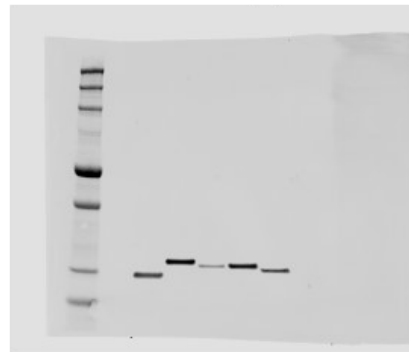
MPTA-17, Anti-GAL4 (1)



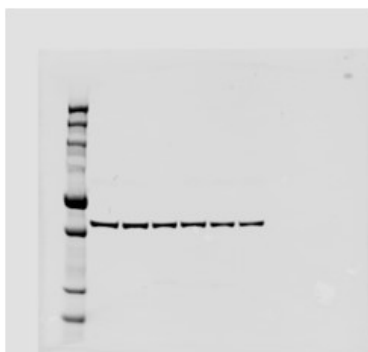
MPTA-17, Anti-GAL4 (2)



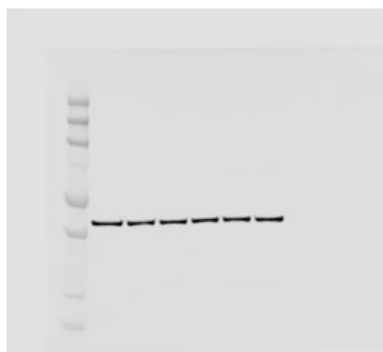
MPTA-17, Anti-GAL4 (3)



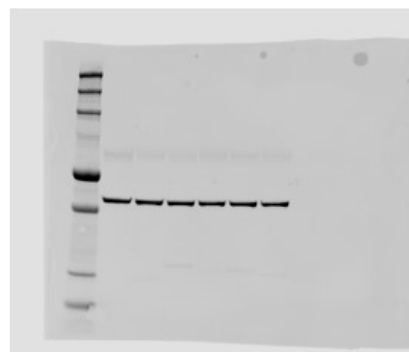
MPTA-17, Anti- $\beta$ -actin (1)



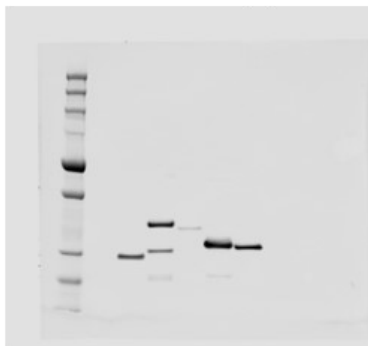
MPTA-17, Anti- $\beta$ -actin (2)



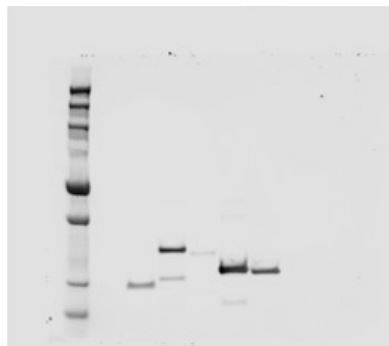
MPTA-17, Anti- $\beta$ -actin (3)



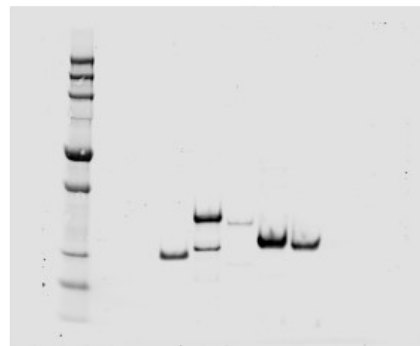
MPTA-45, Anti-GAL4 (1)



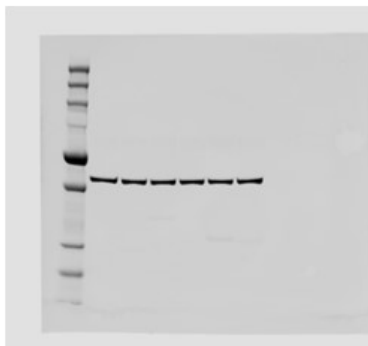
MPTA-45, Anti-GAL4 (2)



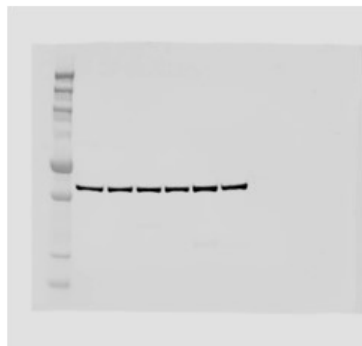
MPTA-45, Anti-GAL4 (3)



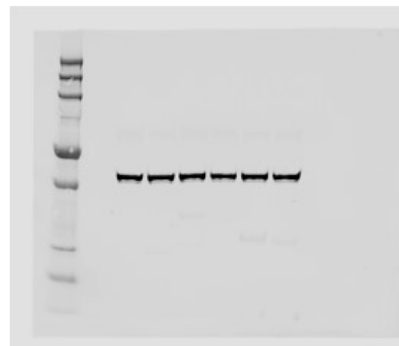
MPTA-45, Anti- $\beta$ -actin (1)



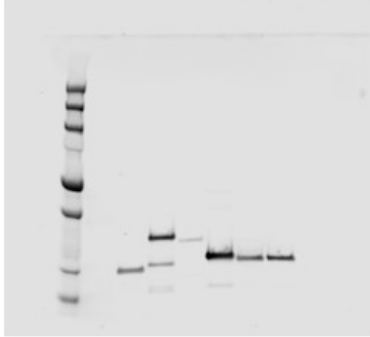
MPTA-45, Anti- $\beta$ -actin (2)



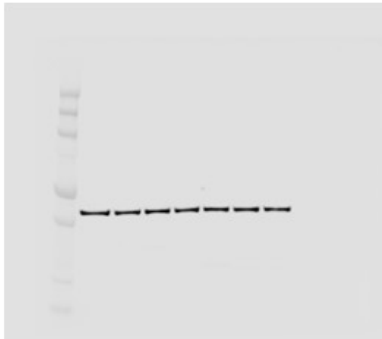
MPTA-45, Anti- $\beta$ -actin (3)



MPTA-45, Anti-GAL4 (Fig. S1)



MPTA-45, Anti- $\beta$ -actin (Fig. S1)



## **Supplementary Table Legends**

**Table S1.** Amino acid composition percent values for the reference human proteome (UniProtKB) and microproteins (Martinez et al., 2020).

**Table S2.** UniProtKB Entry and Gene names corresponding to the polyalanine protein subset.

**Table S3.** GO overrepresentation test results.

**Table S4.** List of the microprotein sequences containing 6-ala, MPTA-1 to MPTA-58, their length and chromosome coordinates (Martinez et al., 2020).

**Table S5.** DualGlo transactivation assay activity values with respective plate controls for MPTA-1 to MPTA-58.

**Table S6.** DualGlo transactivation assay activity values with respective plate controls, and quantification of Western blot for MPTA-17 variants.

**Table S7.** DualGlo transactivation assay activity values with respective plate controls, and quantification of Western blot for MPTA-45 variants.

**Table S8.** DualGlo transactivation assay data processed from raw data for one experiment shown as an example (refer to Experimental).