SUPPORTING INFORMATION: SUPPLEMENTAL FIGURES

Multiplexing engineered receptors for multiparametric evaluation of environmental ligands

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Supplemental Figures

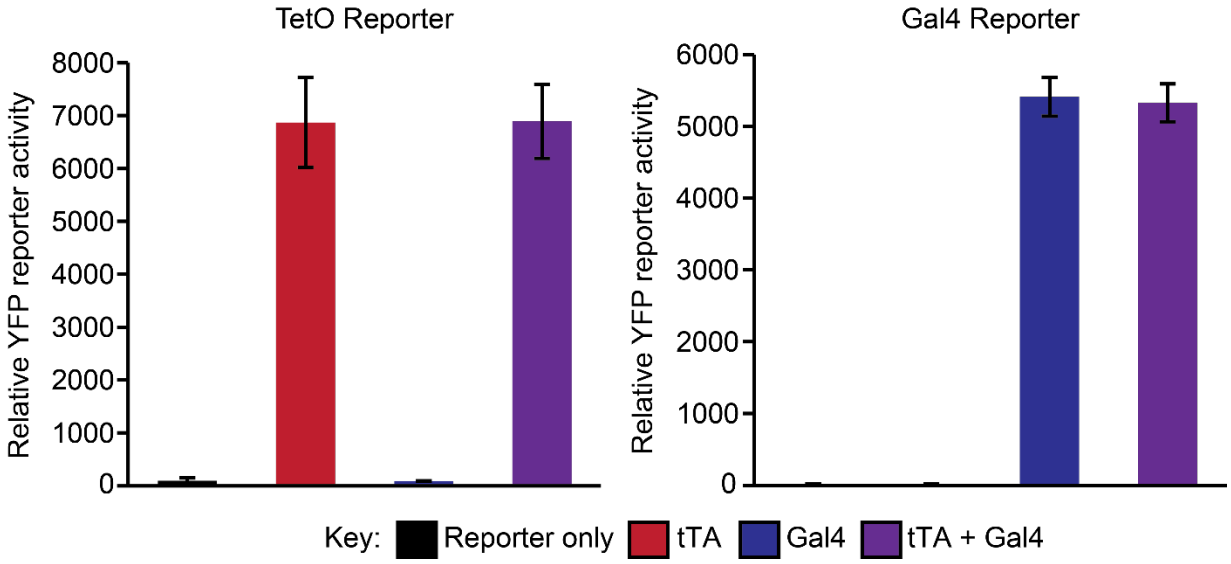


Figure S1: Evaluation of cross-talk between tTA and Gal4 transcription factors and reporter constructs. Soluble tTA and Gal4 transcription factors were transfected individually and in combination in HEK293FT cells, and after 36 h, the reporter activity for a TetO- or Gal4-based reporter (pT_7 or pU_5, respectively) was quantified by flow cytometry. Experiments were conducted in biological triplicate, and error bars indicate one standard deviation. Data were analyzed as in Figure 1.

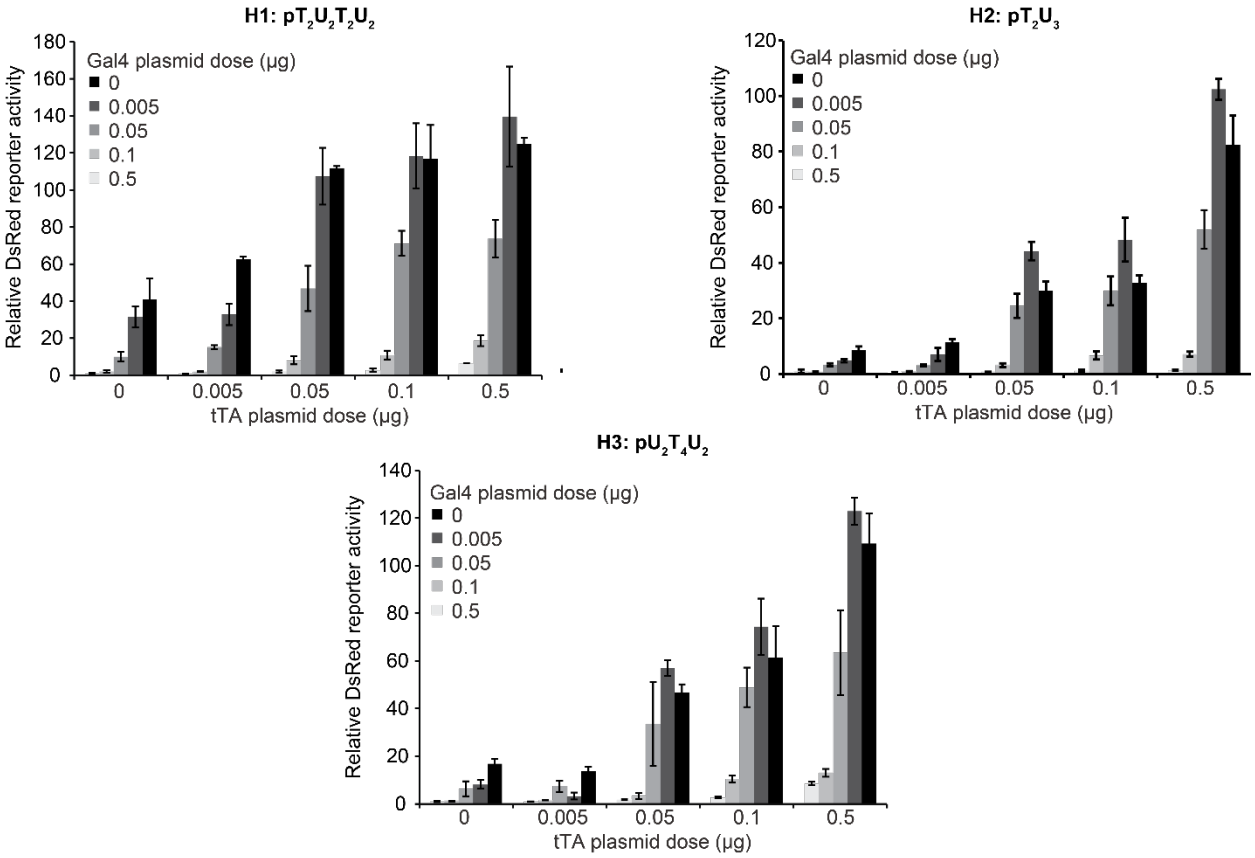
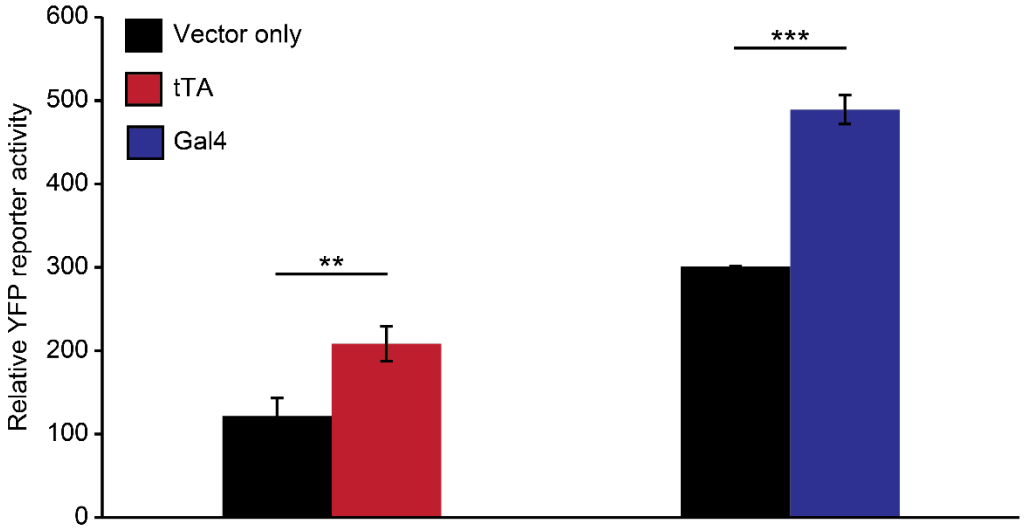


Figure S2: Evaluation of hybrid reporters with soluble transcription factors. Three reporters were evaluated in combination with a large range of tTA and Gal4 plasmid doses (these assays were conducted in 24 well plates, with 1.5E5 cells/well). DsRed reporter activity was quantified by flow cytometry 36 h post-transfection. Experiments were conducted in biological triplicate, and error bars indicate one standard deviation. Data were analyzed as in Figure 1.



TC	Rap-MESA tTA	Rap-MESA Gal4
PC	Rap-MESA Tev	Rap-MESA Tev
Reporter	TetO-YFP	UAS-YFP

Figure S3: Assessment of Rap-MESA signaling with tTA and Gal4 transcription factors. Rap-MESA receptors were constructed to release either a tTA or Gal4 transcription factor and were transfected at a 1 to 1 ratio with a Rap-MESA PC. tTA release was monitored by a tTA responsive TetO-YFP promoter (pT_7), and Gal4 release was monitored by a Gal4 responsive UAS-YFP promoter (pU_5). Rapamycin was added 12 h post-transfection, and YFP reporter activation was monitored by flow cytometry 36 h post-transfection. Experiments were conducted in biological triplicate, and error bars indicate one standard deviation. Data were analyzed as in Figure 1. Statistical comparisons were performed using a two-tailed Student's t-test (**$p \leq 0.01$, ***$p \leq 0.001$).

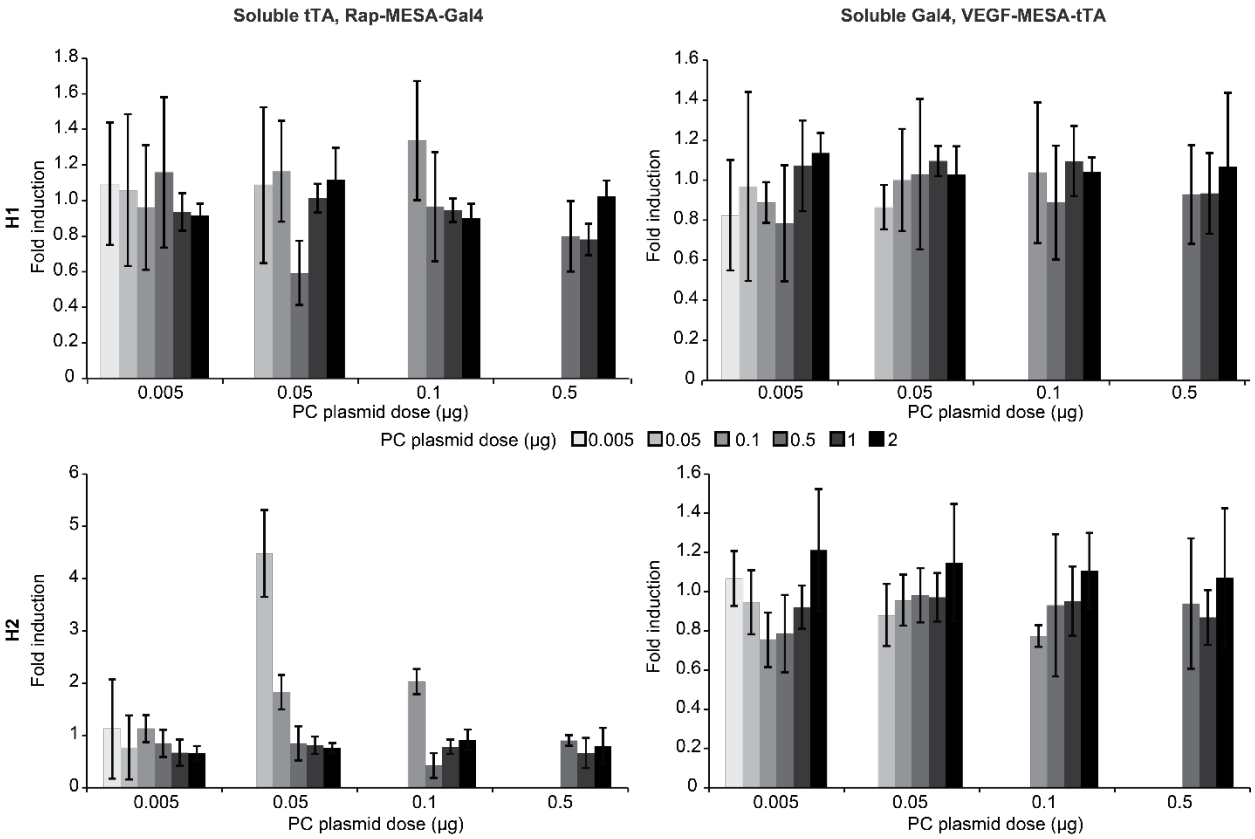


Figure S4: Effect of varying MESA plasmid dose on hybrid reporter activation. Rap-MESA and VEGF-MESA were transfected individually in combination with a soluble transcription factor. Rapamycin or VEGF was added 12 h post-transfection and hybrid reporter activation was assessed by flow cytometry 36 h post-transfection. Conditions where the dose of PC was higher than the dose of TC were not evaluated. Experiments were conducted in biological triplicate, and error bars indicate one standard deviation. Data were analyzed as in Figure 1.

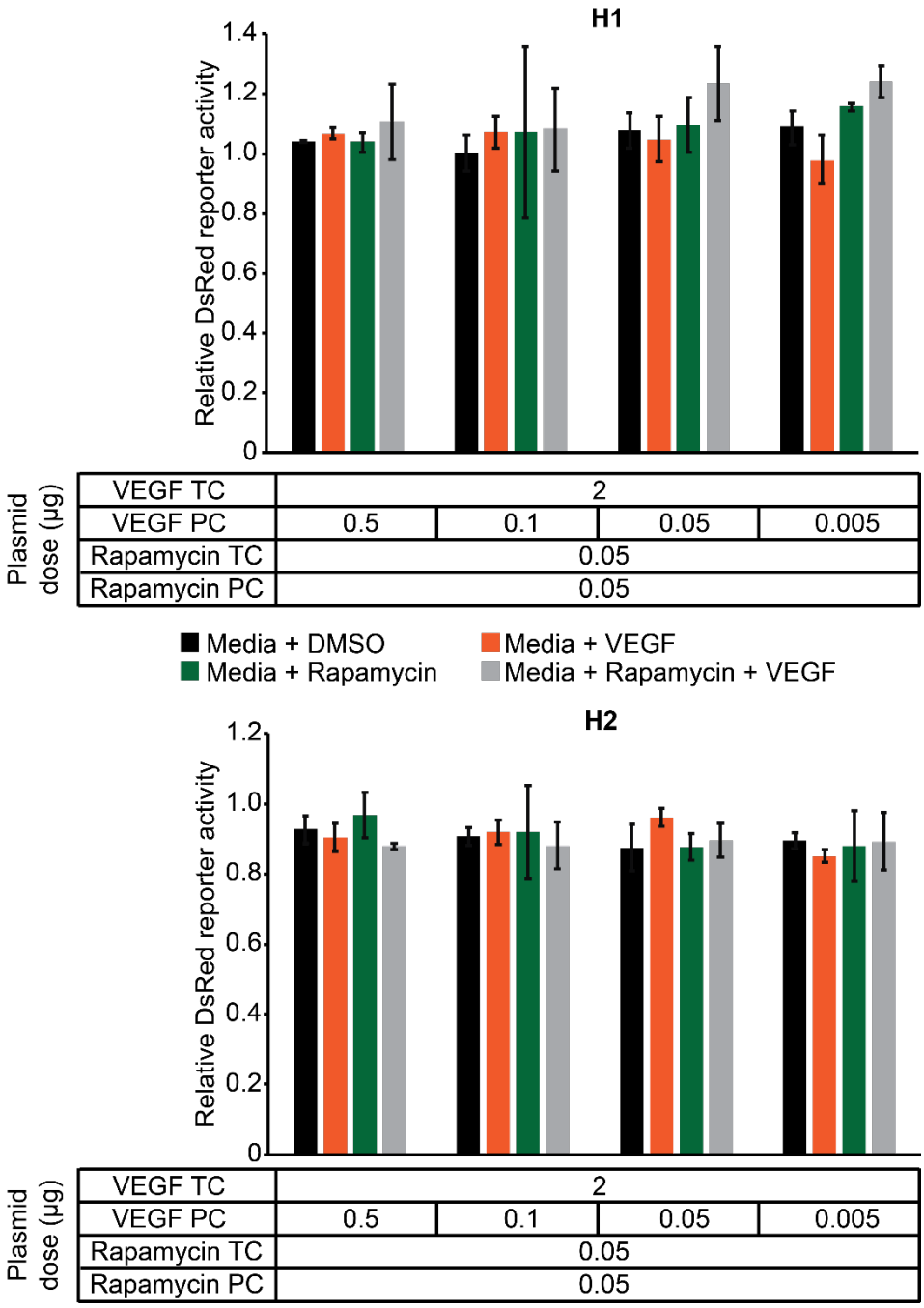


Figure S5: Multiplexed activation of hybrid promoters. Two hybrid promoters were co-transfected with Rap-MESA (TC and PC plasmid dose of 0.05 μg per chain) and VEGF-MESA (TC plasmid dose of 2 μg and a range of PC plasmid doses from 0.005-0.5 μg) to investigate multiplexed MESA activation. The ligands (VEGF and rapamycin) were added to cells 12 h post-transfection, and reporter activation was assessed by flow cytometry 36 h post-transfection. Experiments were conducted in biological triplicate, and error bars indicate one standard deviation. Data were analyzed as in Figure 1.