

Supplementary Data 2

General SP Library Structure and Supplementary Tables 1-4

A) General structure of SP containing plasmids: **SP ([2-5xTFBS]-minP)-GOI-SV40pA**

SP General Design (5x TFBS):

GATCTTTGTTTTGATTAA(**TFBS**)CGCGATGTACGTAAG(**TFBS**)TGCTGATAAG(**TFBS**)AG
ACGTGGCAGTTAG(**TFBS**)GGGGATTAAG(**TFBS**)GCGATTAAGCTTTCCAATATGC(**minP**)
CTAGTACTACTACCAGATAGCTTGGTACCGAGCTCG[G/A]ATCCAGCCACCGTTTAATTA
AACC-**GOI-SV40pA**.

Unbold: spacers; [G/A]: YB_TATA minP=G, others minP=A.

SP Control General Design (No TFBS):

GCTAGAGCTTTCCAATATGC(**minP**)CTAGTACTACTACCAGATAGCTTGGTACCGAGCTC
G[G/A]ATCCAGCCACCGTTTAATTAACCC-**GOI-SV40pA**.

Unbold: spacers, [G/A]: YB_TATA minP=G, others minP=A.

Supplementary Table 1. Library of SP sensor plasmids used in this study

Plasmid - SPs Characterization	TF Target	TFBS (rep) (Supp. Table 5)	minP (Supp. Table 6)	GOI (Supp. Table 6)
pSP01	NA	No TFBS	YB_TATA	mCherry
pSP02	NA	No TFBS	miniCMV	mCherry
pSP03	NA	No TFBS	miniTK	mCherry
pSP04	NA	No TFBS	lateADEp	mCherry
pSP05	IRF4/BATF	IL10P1 (5x)	YB_TATA	mCherry
pSP06	IRF4/BATF	IL10P1 (5x)	miniCMV	mCherry
pSP07	IRF4/BATF	IL10P1 (5x)	miniTK	mCherry
pSP08	IRF4/BATF	IL10P1 (5x)	lateADEp	mCherry
pSP09	IRF4/BATF	IL10P2 (5x)	YB_TATA	mCherry
pSP10	IRF4/BATF	IL10P2 (5x)	miniCMV	mCherry
pSP11	IRF4/BATF	IL10P2 (5x)	miniTK	mCherry
pSP12	IRF4/BATF	IL10P2 (5x)	lateADEp	mCherry
pSP13A	IRF4/BATF	IL10P3 (3x)	YB_TATA	mCherry

pSP14A	IRF4/BATF	IL10P3 (3x)	miniCMV	mCherry
pSP15A	IRF4/BATF	IL10P3 (3x)	miniTK	mCherry
pSP16A	IRF4/BATF	IL10P3 (3x)	lateADEp	mCherry
pSP21	IRF4/BATF	AP-1 (5x)	YB_TATA	mCherry
pSP22	IRF4/BATF	AP-1 (5x)	miniCMV	mCherry
pSP23	IRF4/BATF	AP-1 (5x)	miniTK	mCherry
pSP24A	IRF4/BATF	AP-1 (2x)	lateADEp	mCherry
pSP25	IRF4/BATF	CTLA4 (5x)	YB_TATA	mCherry
pSP26	IRF4/BATF	CTLA4 (5x)	miniCMV	mCherry
pSP27	IRF4/BATF	CTLA4 (5x)	miniTK	mCherry
pSP28	IRF4/BATF	CTLA4 (5x)	lateADEp	mCherry
pSP33	MAF	CD69 (5x)	YB_TATA	mCherry
pSP34	MAF	CD69 (5x)	miniCMV	mCherry
pSP35	MAF	CD69 (5x)	miniTK	mCherry
pSP36	MAF	CD69 (5x)	lateADEp	mCherry
pSP37	MAF	SPATA20 (5x)	YB_TATA	mCherry
pSP38	MAF	SPATA20 (5x)	miniCMV	mCherry
pSP39	MAF	SPATA20 (5x)	miniTK	mCherry
pSP40	MAF	SPATA20 (5x)	lateADEp	mCherry
pSP41	MAF	STAT3 (5x)	YB_TATA	mCherry
pSP42	MAF	STAT3 (5x)	miniCMV	mCherry
pSP43	MAF	STAT3 (5x)	miniTK	mCherry
pSP44	MAF	STAT3 (5x)	lateADEp	mCherry

pSP45	MAF	RNF212 (5x)	YB_TATA	mCherry
pSP46	MAF	RNF212 (5x)	miniCMV	mCherry
pSP47	MAF	RNF212 (5x)	miniTK	mCherry
pSP48A	MAF	RNF212 (4x)	lateADEp	mCherry
pSP49	GATA3	GATA3-A (5x)	YB_TATA	mCherry
pSP50	GATA3	GATA3-A (5x)	miniCMV	mCherry
pSP51	GATA3	GATA3-A (5x)	miniTK	mCherry
pSP52	GATA3	GATA3-A (5x)	lateADEp	mCherry
pSP53	GATA3	GATA3-B (5x)	YB_TATA	mCherry
pSP54	GATA3	GATA3-B (5x)	miniCMV	mCherry
pSP55	GATA3	GATA3-B (5x)	miniTK	mCherry
pSP56	GATA3	GATA3-B (5x)	lateADEp	mCherry
pSP57	GATA3	GATA3-C (5x)	YB_TATA	mCherry
pSP58	GATA3	GATA3-C (5x)	miniCMV	mCherry
pSP59	GATA3	GATA3-C (5x)	miniTK	mCherry
pSP60	GATA3	GATA3-C (5x)	lateADEp	mCherry
pSP61	IRF4/BATF	BRAF (5x)	YB_TATA	mCherry
pSP62A	IRF4/BATF	BRAF (2x)	miniCMV	mCherry
pSP62C	IRF4/BATF	BRAF (5x)	miniCMV	mCherry
pSP63	IRF4/BATF	BRAF (5x)	miniTK	mCherry
pSP64	IRF4/BATF	BRAF (5x)	lateADEp	mCherry
pSP65A	IRF4	GAAA (2x)	YB_TATA	mCherry
pSP65B	IRF4	GAAA (5x)	YB_TATA	mCherry

pSP66	IRF4	GAAA (5x)	miniCMV	mCherry
pSP67	IRF4	GAAA (5x)	miniTK	mCherry
pSP68	IRF4	GAAA (5x)	lateADEp	mCherry
pSP69A	IRF4/BATF	IKZF2 (3x)	YB_TATA	mCherry
pSP70A	IRF4/BATF	IKZF2 (3x)	miniCMV	mCherry
pSP71A	IRF4/BATF	IKZF2 (3x)	miniTK	mCherry
pSP72A	IRF4/BATF	IKZF2 (3x)	lateADEp	mCherry
pSP72B	IRF4/BATF	IKZF2 (5x)	lateADEp	mCherry
pSP73	NR4A2	CON1 (5x)	YB_TATA	mCherry
pSP74	NR4A2	CON2 (5x)	YB_TATA	mCherry
pSP75	NR4A2	MSH4 (5x)	YB_TATA	mCherry
pSP76A	NR4A2	MZB1 (3x)	YB_TATA	mCherry
pSP77A	NR4A2	NEG1 (3x)	YB_TATA	mCherry
pSP78	NR4A2	PRD2 (5x)	YB_TATA	mCherry
pSP79A	IKZF2	B4GA (3x)	YB_TATA	mCherry
pSP79B	IKZF2	B4GA (5x)	YB_TATA	mCherry
pSP80A	IKZF2	CCDC (3x)	YB_TATA	mCherry
pSP80B	IKZF2	CCDC (5x)	YB_TATA	mCherry
pSP81	IKZF2	CON1 (5x)	YB_TATA	mCherry
pSP82A	IKZF2	TMEM (3x)	YB_TATA	mCherry
pSP82B	IKZF2	TMEM (5x)	YB_TATA	mCherry
pSP83	EOMES	CON1 (5x)	YB_TATA	mCherry
pSP84	EOMES	CON2 (5x)	YB_TATA	mCherry

pSP86A	EOMES	CXCR5 (4x)	YB_TATA	mCherry
pSP87	EOMES	HAS3 (5x)	YB_TATA	mCherry
pSP88	EOMES	HEY2A (5x)	YB_TATA	mCherry
pSP89A	EOMES	TMEM9B (4x)	YB_TATA	mCherry
pSP89B	EOMES	TMEM9B (5x)	YB_TATA	mCherry

B) General structure of other plasmids containing SPs cloned using MTK: SP ([5xTFBS]-minP)-GOI-SV40pA

SP General Design (5x TFBS):

GATCTTTGTTTTGATTAA(TFBS)CGCGATGTACGTAAG(TFBS)TGCTGATAAG(TFBS)AG
ACGTGGCAGTTAG(TFBS)GGGGATTAAAG(TFBS)GCGATTAAAGCTTTCCAACGCGT
(minP)CTAGTACTACTACCAGATAGCTTGGTACCGAGCTCGGATCCAGCCACT-GOI-
SV40pA.

Unbold: spacers; Green: MTK2a (5x TF-BSs); Blue: MTK2b (minP YB_TATA+spacer)
(Supplementary Table 6).

SP Control General Design (No TFBS):

GATTAAGCTTTCCAACGCGT(minP)CTAGTACTACTACCAGATAGCTTGGTACCGAGCTC
GGATCCAGCCACT-GOI-SV40pA.

Unbold: spacers; Green: MTK2a (No TF-BSs); Blue: MTK2b (minP YB_TATA+spacer)
(Supplementary Table 6).

Supplementary Table 2. Library of SP sensor plasmids used in this study				
Plasmid - Logic AND/OR Gates	TF Target	TFBS (rep) (Supp. Table 5)	minP (Supp. Table 6)	GOI (Supp. Table 6)
TU1 and TU6	NR4A2 (SP73)	CON1 (5x) (MTK2a)	YB_TATA (MTK2b)	N7-VP16
TU2	MAF (SP45)	RNF212 (5x) (MTK2a)	YB_TATA (MTK2b)	GAL4-N8
TU3	NR4A2 (SP73)	CON1 (5x) (MTK2a)	YB_TATA (MTK2b)	GFP1-10
TU4	MAF (SP45)	RNF212 (5x) (MTK2a)	YB_TATA (MTK2b)	GFP11-βActin
TU8	MAF (SP45)	RNF212 (5x) (MTK2a)	YB_TATA (MTK2b)	N7-VP16

Plasmid - CASwitch Applications	TF Target	TFBS (rep) (Supp. Table 5)	minP (Supp. Table 6)	GOI (Supp. Table 6)
pGB_063	NA	No TF-BSs (MTK2a)	YB_TATA (MTK2b)	rtTA3G
pGB_064	MAF (SP45)	RNF212 (5x) (MTK2a)	YB_TATA (MTK2b)	rtTA3G
pGB_100	MAF (SP45)	RNF212 (5x) (MTK2a)	YB_TATA (MTK2b)	EGFP
pGB_101	MAF (SP45)	RNF212 (5x) (MTK2a)	YB_TATA (MTK2b)	EGFP-DR
Plasmid - Actuator Release	TF Target	TFBS (rep) (Supp. Table 5)	minP (Supp. Table 6)	GOI (Supp. Table 6)
pGB_090	NA	No TF-BSs (MTK2a)	YB_TATA (MTK2b)	IL12p70
pGB_091	NR4A2 (SP73)	CON1 (5x) (MTK2a)	YB_TATA (MTK2b)	IL12p70
Plasmid – Benchmark Comparison	TF Target	TFBS (rep) (Supp. Table 5)	minP (Supp. Table 6)	GOI (Supp. Table 6)
pSP_91	NR4A2	Guo et al., 2024 (8x)	lateADEp	mCherry

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30 **C) Transcription Factor plasmids used in this study**

31 All TF plasmids contain as backbone pCDNA3.1(+) and CMV as promoter. Fluorescent TF
32 reporter constructs were also generated for all TFs in this study (except for MAF) by linking it
33 to EGFP through a self-cleaving P2A peptide (except for BATF which was linked to EBFP).

Supplementary Table 3. Transcription Factor plasmids used in this study				
Plasmid – TFs	TF	Source	UniProt ID	Notes
pCDNA3.1(+)- IRF4 (COL5)	IRF4	Addgene pDONR223- IRF4-WT,	Q15306-1	IRF4 was ordered from Addgene and subcloned in pCDNA3.1(+)-BATF, replacing BATF

		#81782 and this paper		
pCDNA3.1-mBTF	BATF	Addgene #34575	Q16520	-
pcDNA3.1(+)-MAF-FLAG	MAF	Genscript OHu31519	O75444-2	-
pCDNA3.1-GATA3	GATA3	Addgene #115271	P23771-2	-
pcDNA3.1-NE tag-hNurr1	NR4A2	Addgene #102363	P43354-1	-
pcDNA3-IKZF2-V2-V5	IKZF2	Addgene #107391	Q9UKS7-2	-
pcDNA3.1(+)-EOMES	EOMES	Origene pCMV6-EOMES, SC337262 and this paper	O95936-4	EOMES was ordered originally from Origene and subcloned in an pCDNA3.1(+) plasmid under CMV promoter

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35 **D) Other plasmids used in this study**

Supplementary Table 4. Other plasmids used in this study		
Plasmid	Promoter (Supp. Table 6)	GOI (Supp. Table 6)
TU7	shEF1a	GAL4-N8
pLR33	UAS	EBFP

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