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SUPPLEMENTARY INFORMATION

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Generalized extracellular molecule sensor platform for programming cellular behavior

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Supplementary Table 1: P-Values, t-values and degrees of freedom for two-sided T-tests

Figure	Receptor	Compared groups (no adjustments were made for multiple comparisons)	Fold change	P-Value:	Welch-corrected t-values (t) and degrees of freedom (df)
2c	JAK/STAT-GEMS$_{RR120}$	0 ng vs. 1 ng RR120 / mL	5.4	1.848×10^{-6}	t = 8.97 df = 11.25
2c	JAK/STAT-GEMS$_{RR120}$	0 ng vs. 100 ng RR120 / mL	45	2.543×10^{-10}	t = 35.39 df = 8.27
2c	MAPK-GEMS$_{RR120}$	0 ng vs. 1 ng RR120 / mL	9.5	3.343×10^{-7}	t = 15 df = 8.11
2c	MAPK-GEMS$_{RR120}$	0 ng vs. 100 ng RR120 / mL	22	$<1 \times 10^{-15}$	t = 143.1 df = 9.90
2d	VEGFR2-GEMS$_{RR120}$ (NFAT reporter)	0 ng vs. 100 ng RR120 / mL	21	3.69×10^{-7}	t = 13.86 df = 8.56
2d	VEGFR2-GEMS$_{RR120}$ (NF-κB reporter)	0 ng vs. 100 ng RR120 / mL	5.2	1.242×10^{-7}	t = 13.16 df = 9.99
2d	VEGFR2-GEMS$_{RR120}$) (MAPK reporter)	0 ng vs. 100 ng RR120 / mL	6.4	8.056×10^{-8}	t = 14.03 df = 9.82
3a	JAK/STAT-GEMS$_{nicotine}$	0 µM vs. 0.1 µM Nicotine	1.6	3.485×10^{-8}	t = 10.02

					df = 15.56
3a	JAK/STAT-GEMS$_{nicotine}$	0 µM vs. 0.33 µM Nicotine	2.2	7.731×10^{-11}	t = 16.97 df = 14.22
3a	JAK/STAT-GEMS$_{nicotine}$	0 µM vs. 10 µM Nicotine	3.4	$<1 \times 10^{-15}$	t = 35.82 df = 15.23
3a	MAPK-GEMS$_{nicotine}$	0 µM vs. 0.1 µM Nicotine	2.5	4.775×10^{-12}	t = 19.73 df = 14.82
3a	MAPK-GEMS$_{nicotine}$	0 µM vs. 0.33 µM Nicotine	5.5	2.595×10^{-10}	t = 27.92 df = 9.33
3a	MAPK-GEMS$_{nicotine}$	0 µM vs. 10 µM Nicotine	14	6.3×10^{-13}	t = 61.27 df = 8.84
3b	JAK/STAT-GEMS$_{SunTag}$	0 % vs. 0.002 % (v/v) bacterial lysate (SunTag)	6.9	1.246×10^{-9}	t = 21.74 df = 8.01
3b	JAK/STAT-GEMS$_{SunTag}$	0 % vs. 0.02 % (v/v) bacterial lysate (SunTag)	30	2.086×10^{-8}	t = 23.01 df = 15.58
3b	MAPK-GEMS$_{SunTag}$	0 % vs. 0.002 % (v/v) bacterial lysate (SunTag)	2.0	1.9×10^{-13}	t = 23.01 df = 15.58
3b	MAPK-GEMS$_{SunTag}$	0 % vs. 0.02 % (v/v) bacterial lysate (SunTag)	2.4	2×10^{-15}	t = 30.73 df = 15.62

4a	JAK/STAT-GEMS$_{PSA}$	0 ng vs. 1 ng	2.7	1.711×10^{-4}	t = 6.16 df = 8.94
4a	JAK/STAT-GEMS$_{PSA}$	1 ng vs. 2 ng PSA/mL	2.1	5.018×10^{-7}	t = 8.54 df = 14.45
4a	JAK/STAT-GEMS$_{PSA}$	2 ng vs. 4 ng PSA/mL	2.2	1.206×10^{-7}	t = 12.44 df = 10.57
4a	JAK/STAT-GEMS$_{PSA}$	4 ng vs. 6 ng PSA/mL	1.3	1.207×10^{-5}	t = 6.23 df = 16
4a	JAK/STAT-GEMS$_{PSA}$	6 ng vs. 10 ng PSA/mL	1.4	1.763×10^{-4}	t = 5.55 df = 10.94
4a	JAK/STAT-GEMS$_{PSA}$	10 ng vs. 20 ng PSA/mL	1.4	3.617×10^{-6}	t = 7.12 df = 14.92
4a	JAK/STAT-GEMS$_{PSA}$	0 ng vs. 40 ng PSA/mL	32	7.487×10^{-10}	t = 32.6 df = 8.07
4b	MAPK-GEMS$_{PSA}$	0 ng vs. 0.1 ng PSA/mL	2.7	1.019×10^{-6}	t = 11.4 df = 9.16
4b	MAPK-GEMS$_{PSA}$	0.1 ng vs. 0.33 ng PSA/mL	2.7	2.450×10^{-10}	t = 16.63 df = 13.41
4b	MAPK-GEMS$_{PSA}$	0.33 ng vs. 1 ng PSA/mL	2.2	7.517×10^{-10}	t = 17.55

					df = 11.86
4b	MAPK-GEMS$_{PSA}$	1 ng vs. 4 ng PSA/mL	1.3	2.837×10^{-7}	t = 8.71 df = 15.11
4b	MAPK-GEMS$_{PSA}$	0 ng vs. 40 ng PSA/mL	22	1.536×10^{-8}	t = 22.5 df = 8.03
4c	MAPK-GEMS$_{PSA}$	10 % (v/v) PSA negative vs. patient 1 serum	2.0	5.238×10^{-9}	t = 23.59 df = 8.45
4c	MAPK-GEMS$_{PSA}$	10 % (v/v) PSA negative vs. patient 2 serum	4.2	1.018×10^{-7}	t = 14.04 df = 9.60
4c	MAPK-GEMS$_{PSA}$	10 % (v/v) PSA negative vs. patient 3 serum	10	8.678×10^{-9}	t = 24.03 df = 8.06
5a	MAPK-GEMS$_{RR120}$ (Y677F) + JAK/STAT-GEMS$_{SunTag}$	0 ng vs. 100 ng RR120 / mL	9.3 (MAPK reporter)	8.556×10^{-11}	t = 34.07 df = 8.97
5a	MAPK-GEMS$_{RR120}$ (Y677F) + JAK/STAT-GEMS$_{SunTag}$	0 ng vs. 100 ng RR120 / mL	1.3 (STAT3 reporter)	0.012	t = 2.95 df = 12.02
5a	MAPK-GEMS$_{RR120}$	0 % vs. 0.002 % (v/v) bacterial	1.3	0.038	t = 2.36

	(Y677F) + JAK/STAT-GEMS$_{SunTag}$	lysate (SunTag)	(MAPK reporter)		df = 12.01
5a	MAPK-GEMS$_{RR120}$ (Y677F) + JAK/STAT-GEMS$_{SunTag}$	0 % vs. 0.002 % (v/v) bacterial lysate (SunTag)	12.9 (STAT3 reporter)	2.759×10^{-11}	t = 43.83 df=8.47
5a	MAPK-GEMS$_{RR120}$ (Y677F) + JAK/STAT-GEMS$_{SunTag}$	No inducer vs. 100 ng RR120 / mL + 0.002 % (v/v) bacterial lysate (SunTag)	7.4 (MAPK reporter)	5.256×10^{-9}	t = 22.49 df=8.69
5a	MAPK-GEMS$_{RR120}$ (Y677F) + JAK/STAT-GEMS$_{SunTag}$	No inducer vs. 100 ng RR120 / mL + 0.002 % (v/v) bacterial lysate (SunTag)	14.9 (STAT3 reporter)	8.186×10^{-8}	t = 18.11 df=8.057
5b	WT WEN1.3 cells	0 ng vs. 100 ng RR120 / mL	1.1	0.377	t = 0.91 df = 14.18
5b	JAK/STAT-GEMS$_{RR120}$ WEN1.3 cells (polyclonal cell line)	0 ng vs. 100 ng RR120 / mL	1.5	3.229×10^{-5}	t = 6.09 df = 13.52
5b	MAPK-GEMS$_{RR120}$ WEN1.3 cells (polyclonal cell line)	0 ng vs. 100 ng RR120 / mL	2.4	5.110×10^{-10}	t = 13.27 df = 15.9

Supplementary Information

Supplementary Table 2: Plasmid Table

Plasmid	Description and cloning strategy	Reference / Source
hKDR	Mammalian VEGFR2-tGFP expression vector (P_{hCMV}-VEGFR2-tGFP -pA) (Addgene no. 83436)	Raikwar et al.[1]
MKp37	Mammalian TetR-ELK1 fusion protein expression vector (P_{hCMV}-TetR-ELK1-pA)	Keeley et al.[2]
pANT7_cGST IL6-ST cDNA	In vitro interleukin 6 signal transducer (IL-6RB) cDNA expression vector (P_{T7}-IL-6RB_cDNA-cGST-pA) (clone HsCD00403511)	DNASU, Center for Personalized Diagnostics (unpublished)
pCDNA3.1(+)	Mammalian expression vector (P_{hCMV}-MCS-pA)	Life Technologies, CA
pCMV(CAT)T7-SB100	Mammalian sleeping beauty transposase expression vector (P_{hCMV}-SB100X-pA) (Addgene no. 34879)	Mates et al.[3]
pCMV sport6 EpoR	EpoR cDNA expression vector (P_{hCMV}-mouse EpoR_cDNA-pA) (clone IRAVp968H0490D)	Source BioScience UK Limited
pDONR223-FGFR1	Cloning vector for human FGFR1 (recombination site-FGFR1- recombination site) (Addgene no. 23922)	Johannessen et al.[4]
pDONR201_IL6R	Cloning vector for human IL-6RA (recombination site- IL-6RA - recombination site) (clone HsCD00001372)	DNASU, Harvard Institute of Proteomics (unpublished)

pET MBP mCherry LIC cloning vector (MBP-mCherry)	Lactose-inducible bacterial MBP-mCherry fusion expression vector (P_{T7}-TetO-MBP-mCherry-$T7_{term}$) (Addgene no. 29747)	Gift from Scott Gradia (unpublished)
pHRdSV40-dCas9-10xGCN4_v4-P2A-BFP	Mammalian lentiviral SunTag Cas9 expression vector (5'UTR-P_{SV40}-Cas9-SunTag-P2A-BFP-3'UTR) (Addgene no. 60903)	Tanenbaum et al.[5]
pHRdSV40-scFv-GCN4-sfGFP-VP64-GB1-NLS	Mammalian scFv-VP64 fusion expression vector (5'UTR-P_{SV40}-scFv-sfGFP-VP64-GB1-NLS-3'UTR) (Addgene no. 60904)	Tanenbaum et al.[5]
Proinsulin-NanoLuc in pLX304	Lentiviral expression vector for constitutive of NanoLuc-tagged insulin expression (Addgene no. 62057)	Burns et al.[6]
pSBbi-BP	Vector backbone containing a P_{hEF1a} promoter, SfiI cleavage sites, BFP and puromycin resistance gene expression and ITR regions for transposon mediated stable cell line generation (ITR-P_{hEF1a}-SfiI-pA, P_{RPBSA}-PuroR-P2A-BFP-PA-ITR) (Addgene no. 60512)	Kowarz et al.[7]
rOpto-mFGFR1_333	Mammalian $FGFR1_{int}$-CPH1SSo fusion expression vector (P_{hCMV}-mFGFR1-CPH1So-pA) (Addgene no. 82682)	Reichhart et al.[8]
pAT13	Mammalian PIP-ELK1 expression vector (P_{hCMV}-PIP-ELK1-pA)	Tastanova et al. (unpublished) GenBank accession no. MG757108
pDF145	In vitro RNA production vector (P_{T7}-SpAH-Env140ac)	Fuchs et al.[9]
pHY30	Mammalian reporter plasmid for NFAT-induced SEAP expression (O_{NFAT}-$P_{hCMVmin}$-SEAP-pA)	Ye et al.[10]

pKR32	Mammalian reporter plasmid for NF-κB-induced SEAP expression ($O_{NF\text{-}\kappa B}$-$P_{hCMVmin}$-SEAP-pA)	Schukur et al.[11]
pLeo26	Mammalian expression vector (P_{SV40}-MCS-pA)	Kojima et al. [12]
pLeo49	Mammalian secreted FRB expression vector (P_{hCMV}-SP-FRB-MCS-pA)	Kojima et al. [12]
pLeo50	Mammalian secreted FKBP expression vector (P_{hCMV}-SP-FKBP-MCS-pA)	Kojima et al. [12]
pLS13	Mammalian reporter plasmid for STAT3-induced SEAP expression (O_{Stat3}-$P_{hCMVmin}$-SEAP-pA)	Schukur et al.[11]
pLS15	Mammalian STAT3 expression vector (P_{hCMV}-STAT3-pA)	Schukur et al.[11]
pMF111	Mammalian reporter plasmid for TetR-Elk1 induced SEAP expression (O_{TetR}-$P_{hCMVmin}$-SEAP-pA)	Fussenegger et al.[13]
pMF199	Mammalian reporter plasmid for PIP-Elk1 induced SEAP expression (O_{PIP}-$P_{hCMVmin}$-SEAP-pA)	Fussenegger et al.[13]
pMM1	Mammalian expression vector with a modified MCS (P_{hCMV}-MCS-pA; MCS, EcoRI-ATG-SpeI-NheI-BamHI- STOP-XbaI-HindIII-FseI-pA).	Müller et al. [14]
pMM585	Mammalian SP-MCS expression vector (P_{hCMV}-SP-MCS-pA). The sequence of SP-MCS is gaattccaccATGACTAGTGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGCTAGTGCTAGCGGATCCACCGGTGTCTAGaaagctt and contains (among others) cleavage sites for NheI and BamHI. The complete sequence of flanking regions and the backbone is provided in plasmid pTS1012.	Müller et al. (unpublished)
pLeo127	Lactose-inducible bacterial mCherry-mCherry fusion expression vector. (P_{T7}-TetO-mCherry-mCherry-$T7_{term}$) mCherry was amplified with OLeo295 (aattaatctagaaataattttgtttaactttaagaaggagatatagcatgGTGAGCAAGGGCGAGGAG) and OLeo267 (aattaaggatccCTTGTACAGCTCGTCCATGCC) and cloned into pET MBP mCherry LIC cloning vector (MBP-mCherry) restricted with XbaI/BamHI.	This work (GenBank accession no. MG437006)
pLeo410	Lactose-inducible bacterial SunTag-mCherry expression vector. (P_{T7}-mCherry-$SunTag_{8\times}$-$T7_{term}$) SunTag was amplified from pHRdSV40-dCas9-10xGCN4_v4-P2A-BFP with OLeo320	This work (GenBank accession no.

	(aattaatgtacaagGTGGACGGCATTGGTAGTGGGA, BamHI 3' of the binding site) and OLeo315 (aattaagcggccgcttAGCCCTTTTTGAGCCTAGCAACTTCG) and cloned into pLeo127 restricted with BamHI/NotI.	MG437007)
pLeo614	Mammalian secreted FKBP vector with additional cloning sites (P_{hCMV}-FseI-SP-BamHI-FKBP-MCS-pA) FKBP was amplified from pLeo50 with OLeo301 (ccaagctagcggccggccaccatggaaactgatactttgctgctctgggtcctgctgctgtgggtccctggatcaacgggggacggatcCATGGGCGTTCAGGTTGAAACC) and OLeo100 (tctagAGAATTCTTCCAGTTTCAGCAGTTCC) and cloned into pCDNA3.1 restricted with NheI/EcoRI	This work (GenBank accession no. MG437008)
pLeo615	Mammalian VHH_{A52}-$EpoR_{3A}$-IL-$6RB_{int}$ receptor expression vector (P_{hCMV}-SP-VHH_{A52}-$EpoR_{3A}$-IL-$6RB_{int}$-pA) The intracellular domain of pTS1008 was amplified with OLeo180 (aaccaagaattcGCACCTTCACCCAGCCTC) and OLeo107 (aaccaagcggccgcctaCTGAGGCATGTAGCCGCC) and restricted with EcoRI/NotI, VHH_{A52} was ordered as IDT gBlock and was restricted with BamHI/EcoRI. Both fragments were cloned into pLeo614 restricted with BamHI/NotI.	This work (GenBank accession no. MG437009)
pLeo617	Mammalian VHH_{A52}-$EpoR_{3A}$-IL-$6RB_{int}$ receptor expression vector (P_{SV40}-SP-VHH_{A52}-$EpoR_{3A}$-IL-$6RB_{int}$-pA) pLeo615 was restricted once with NheI/EcoRI and once with EcoRI/NotI. Both fragments were cloned into pLeo26 restricted with NheI/NotI.	This work (GenBank accession no. MG437010)
pLeo618	Mammalian VHH_{A52}-$EpoR_{3A}$-IL-$6RB_{m}$ receptor expression vector (P_{SV40}-SP-A52-$EpoR_{3A}$-IL-	This work

	$6RB_m$-pA) The mutation Y759A was introduced into pLeo617 by whole plasmid PCR with OLeo498 (ccaaccgtctcgaagcCTGGACAGTGCTCGAAGTGTTTTG) and OLeo499 (ccaaccgtctcagcTTCTACCGTGGTACACAGTGGC) and restriction with BsmBI.	(GenBank accession no. MG437011)
pLeo619	Mammalian $GEMS_{RR120}$ expression vector (P_{SV40}-SP-VHH_{A52}-$EpoR_m$-IL-$6RB_m$-pA). This framework is referred to as GEMS. The mutation F93A was introduced into pLeo618 by whole plasmid PCR with OLeo377 (GCTGTGCCGCTGGAGCTGCAGG) and OLeo376 (ACTCGATGTGTCCGCTGTTGGC) and phosphorylation of the PCR product for blunt end ligation.	This work (GenBank accession no. MG437012)
pLeo620	Mammalian $GEMS_{SunTag}$ expression vector (P_{SV40}-SP-$scFv_{\alpha GCN4}$-$EpoR_m$-IL-$6RB_m$-pA) The $scFv_{\alpha GCN4}$ was amplified from pHRdSV40-scFv-GCN4-sfGFP-VP64-GB1-NLS with OLeo303 (ccaaggatCCGACATCGTGATGACCCAGAGC) and OLeo322 (aattaagaattctccggAGCTGCTCACGGTCACCAG), and cloned into pLeo619 restricted with BamHI/EcoRI.	This work (GenBank accession no. MG437013)
pLeo621	Mammalian $GEMS_{PSA}$ $scFv_{5d3d11}$ expression vector (P_{SV40}-SP-$scFv_{5d3d11}$-$EpoR_m$-IL-$6RB_m$-pA) The PSA scFv 5d3d11 gBlock was cloned into pLeo619 restricted with BamHI/EcoRI.	This work (GenBank accession no. MG437014)
pLeo622	Mammalian $GEMS_{PSA}$ $scFv_{8g8f5}$ expression vector (P_{SV40}-SP-$scFv_{8g8f5}$-$EpoR_m$-IL-$6RB_m$-pA) The PSA scFv 8g8f5 gBlock was cloned into pLeo619 restricted with BamHI/EcoRI.	This work (GenBank accession no. MG437015)
pLeo623	Mammalian $GEMS_{PSA}$ $scFv_{5a5}$ expression vector (P_{SV40}-SP-$scFv_{5a5}$-$EpoR_m$-IL-$6RB_m$-pA)	This work

	The PSA scFv 5a5 gBlock was cloned into pLeo619 restricted with BamHI/EcoRI.	(GenBank accession no. MG437016)
pLeo626	Mammalian $GEMS_{nicotine}$ $Nic12_{VH}$ expression vector (P_{SV40}-SP-$Nic12_{VH}$-$EpoR_m$-IL-$6RB_m$-pA) The Nic12 V_H gBlock was cloned into pLeo619 restricted with BamHI/EcoRI.	This work (GenBank accession no. MG437017)
pLeo627	Mammalian $GEMS_{nicotine}$ $Nic12_{VL}$ expression vector (P_{SV40}-SP-$Nic12_{VL}$-$EpoR_m$-IL-$6RB_m$-pA) The Nic12 V_L gBlock was cloned into pLeo619 restricted with BamHI/EcoRI.	This work (GenBank accession no. MG437018)
pLeo628	Mammalian MAPK-$GEMS_{RR120}$ expression vector (P_{SV40}-SP-VHH_{A52}-$EpoR_{m0A}$-$FGFR1_{int}$-pA) $FGFR1_{int}$ was amplified from rOpto-mFGFR1_333 with OLeo628 (caaccgtctctgtcCATGAAGAGCGGCACCAAGAAG) and OLeo629 (caacgcggccgcctaGCGCCGTTTGAGTCCACT) and restricted with BsmBI and NotI. $EpoR_m$ was amplified from pLeo619 with OLeo180 (aaccaagaattcGCACCTTCACCCAGCCTC) and OTS172 (gtcacgtctcaGGACAGCAGGGCCAGAACC) and restricted with BsmBI/EcoRI. Both restrictions were cloned into pLeo619 restricted with EcoRI/NotI.	This work (GenBank accession no. MG437019)
pLeo642	Mammalian MAPK-$GEMS_{RR120}$ expression vector with one alanine C-terminal of the $EpoR_m$ transmembrane helix (P_{SV40}-SP-VHH_{A52}-$EpoR_{m1A}$-$FGFR1_{int}$-pA) One alanine was added by whole plasmid PCR of pLeo628 with the primers OLeo630 (caaccgtctctgtccgctATGAAGAGCGGCACCAAGAAG) and OTS172 and restriction with BsmBI.	This work (GenBank accession no. MG437020)
pLeo643	Mammalian MAPK-$GEMS_{RR120}$ expression vector with two alanines C-terminal of the $EpoR_m$	This work

	transmembrane helix (P_{SV40}-SP-VHH_{A52}-$EpoR_{m2A}$-$FGFR1_{int}$-pA) Two alanines were added by whole plasmid PCR of pLeo628 with the primers OLeo631 (caaccgtctctgtccgctgcgATGAAGAGCGGCACCAAGAAG) and OTS172 and restriction with BsmBI.	(GenBank accession no. MG437021)
pLeo644	Mammalian MAPK-$GEMS_{RR120}$ expression vector with three alanines C-terminal of the $EpoR_m$ transmembrane helix (P_{SV40}-SP-VHH_{A52}-$EpoR_{m3A}$-$FGFR1_{int}$-pA) Three alanines were added by whole plasmid PCR of pLeo628 with the primers OLeo632 (caaccgtctctgtccgccgctgcgATGAAGAGCGGCACCAAGAAG) and OTS172 and restriction with BsmBI.	This work (GenBank accession no. MG437022)
pLeo645	Mammalian MAPK-$GEMS_{RR120}$ expression vector with four alanines C-terminal of the $EpoR_m$ transmembrane helix (P_{SV40}-SP-VHH_{A52}-$EpoR_{m4A}$-$FGFR1_{int}$-pA) Four alanines were added by whole plasmid PCR of pLeo628 with the primers OLeo633 (caaccgtctctgtccgccgctgcgGCGATGAAGAGCGGCACCAAGAAG) and OTS172 and restriction with BsmBI.	This work (GenBank accession no. MG437023)
pLeo665	Mammalian reporter plasmid for TetR-Elk1-induced NanoLuc expression (O_{TetR}-$P_{hCMVmin}$-SP-NanoLuc-pA). NanoLuc was excised from pTS1011 with EcoRI/XbaI and cloned into pMF111 restricted with EcoRI/NheI.	This work (GenBank accession no. MG437024)
pLeo667	Mammalian MAPK-$GEMS_{Nicotine}$ $Nic12_{VH}$ expression vector (P_{SV40}-SP-$Nic12_{VH}$-$EpoR_m$-$FGFR1_{int}$-pA) $FGFR1_{int}$ was excised from pLeo628 and cloned into pLeo626 restricted with EcoRI/NotI.	This work (GenBank accession no. MG437025)
pLeo668	Mammalian MAPK-$GEMS_{Nicotine}$ $Nic12_{VL}$ expression vector (P_{SV40}-SP-$Nic12_{VL}$-$EpoR_m$-	This work

	$FGFR1_{int}$-pA) $FGFR1_{int}$ was excised from pLeo628 and cloned into pLeo627 restricted with EcoRI/NotI.	(GenBank accession no. MG437026)
pLeo669	Mammalian MAPK-$GEMS_{SunTag}$ expression vector (P_{SV40}-SP-$ScFv_{GCN4}$-$EpoR_m$-$FGFR1_{int}$-pA) $FGFR1_{int}$ was excised from pLeo628 and cloned into pLeo620 restricted with EcoRI/NotI.	This work (GenBank accession no. MG437027)
pLeo670	Mammalian MAPK-$GEMS_{PSA}$ $scFv_{8g8f5}$ expression vector (P_{SV40}-SP-$scFv_{8g8f5}$-$EpoR_m$-$FGFR1_{int}$-pA) $FGFR1_{int}$ was excised from pLeo628 and cloned into pLeo622 restricted with EcoRI/NotI.	This work (GenBank accession no. MG437028)
pLeo671	Mammalian MAPK-$GEMS_{PSA}$ $scFv_{5a5}$ expression vector (P_{SV40}-SP-$scFv_{5a5}$-$EpoR_m$-$FGFR1_{int}$-pA) $FGFR1_{int}$ was excised from pLeo628 and cloned into pLeo623 restricted with EcoRI/NotI.	This work (GenBank accession no. MG437029)
pLeo690	Mammalian VEGFR2-$GEMS_{RR120}$ expression vector without alanines C-terminal of the $EpoR_m$ transmembrane helix (P_{SV40}-SP-VHH_{A52}-$EpoR_{m0A}$-$VEGFR2_{int}$-pA). The intracellular domain on pLeo619 was removed by whole plasmid PCR with OLeo688 (gatAGCAGGGCCAGAACCG) and OLeo499 (ccaaccgtctcaGCTTCTACCGTGGTACACAGTGGC). VEGFR2's intracellular domain was amplified from hKDR with OLeo709 (gCTACGGACCGTTAAGCGGG) and OLeo710 (aaccatctagaGTTTAAACTCTTTCTTCACCGGCATCTGCATC (NotI cleavage site 3' of the binding site)). Both PCR products were phosphorylated and restricted with NotI for cloning with blunt-end/NotI.	This work (GenBank accession no. MG437030)
pLeo692	Mammalian MAPK-$GEMS_{RR120}$ expression vector with mutated PLCG binding site (P_{SV40}-SP-VHH_{A52}-$EpoR_{m0A}$-$FGFR1_{int}$(Y766F)-pA) The mutation Y766F was introduced into pLeo628 by	This work (GenBank accession no.

	whole plasmid PCR with OLeo679 (aaccgtctcgagttcCTGGACCTGTCCATACCGCT) and OLeo678 (ccaagcggccgcctacgtctcgaCTCCTGGTTGGAGGTCAAGG) and restriction with BsmBI.	MG437031)
pLeo693	Mammalian MAPK-GEMS$_{RR120}$ expression vector with mutated STAT binding site (P$_{SV40}$-SP-VHH$_{A52}$-EpoR$_{m0A}$-FGFR1$_{int}$(Y677F)-pA) The mutation Y677F was introduced into pLeo628 by whole plasmid PCR with OLeo694 (aacacgtctcgaAGATCCGGTCAAACAACGCCTC) and OLeo695 (aacacgtctcatcttCACACACCAGAGCGATGTGTG) and restriction with BsmBI.	This work (GenBank accession no. MG437032)
pLeo694	Mammalian MAPK-GEMS$_{RR120}$ expression vector with ITR regions and P$_{hEF1a}$ for stable integration (ITR-P$_{hEF1a}$-SP-VHH$_{A52}$-EpoR$_{m0A}$-FGFR1$_{int}$-pA, P$_{RPBSA}$-PuroR-P2A-BFP-PA-ITR). MAPK-GEMS$_{RR120}$ was amplified with OLeo623 (cacaaggcctctgaggccAGCTTCGAATCGCGCTAGC) and OLeo540 (caacaggcctgacaggcCGGGTTTAAACGGGCCCTCTAG) and cloned into pSBbi-BP restricted with SfiI.	This work (GenBank accession no. MG437033)
pLeo695	Mammalian JAK/STAT-GEMS$_{RR120}$ expression vector with ITR regions and P$_{hEF1a}$ for stable integration (ITR-P$_{hEF1a}$-SP-VHH$_{A52}$-EpoR$_{m0A}$-IL-6RB$_{m}$-pA, P$_{RPBSA}$-PuroR-P2A-BFP-PA-ITR). JAK/STAT -GEMS$_{RR120}$ was amplified with OLeo623 (cacaaggcctctgaggccAGCTTCGAATCGCGCTAGC) and OLeo540 (caacaggcctgacaggcCGGGTTTAAACGGGCCCTCTAG) and cloned into pSBbi-BP restricted with SfiI.	This work (GenBank accession no. MG437034)
pLeo696	Mammalian IL-6RA expression vector with the same promoter and SP as GEMS (P$_{SV40}$-SP- IL-6RA -pA) IL-6RA was amplified pDONR201_IL6R with OLeo692	This work (GenBank accession no.

	(ccaaggatccGAACTTCTAGATCCATGTGGTTATATCA) and OLeo107 (aaccaagcggccgcctaCTGAGGCATGTAGCCGCC) and cloned into pLeo619 restricted with BamHI/NotI	MG437035)
pLeo697	Mammalian IL-6RB expression vector with the same promoter and SP as GEMS (P_{SV40}-SP- IL-6RB -pA) IL-6RB was amplified from pANT7_cGST IL6-ST with OLeo693 (aattggatccCCGGGAGCGGCGCTG) and OLeo105 (aaccaagcggccgcctaTCTGGGGAAGAAGTAGTCTGTATTGC) and cloned into pLeo619 restricted with BamHI/NotI	This work (GenBank accession no. MG437036)
pLeo698	Mammalian FGFR1 expression vector with the same promoter and SP as GEMS (P_{SV40}-SP-FGFR1-pA) FGFR1 was amplified from pDONR223-FGFR1 with OLeo703 (cacaggatccAGGCCGTCCCCGACCTTGC) and OLeo699 (aacagcggccgcTCAGCGGCGTTTGAGTCCGCC) and cloned into pLeo619 restricted with BamHI/NotI	This work (GenBank accession no. MG437037)
pTS1000	Mammalian FRB-EpoR_{0A}-IL-6RB_{int} receptor expression vector (P_{hCMV}-SP-FRB-EpoR_{0A}-IL-6RB_{int}-pA) EpoR_{0A} was amplified from pCMV sport6 EpoR with OTS171 (gtcagaattcGCACCTTCACCCAGCCTC) and OTS172 (gtcacgtctcAGGACAGCAGGGCCAGAACC) and restricted with EcoRI/ BsmBI. IL-6RB_{int} was amplified from pANT7_cGST IL6-ST with OTS173 (gtcacgtctcagtccAATAAGCGAGACCTAATTAAAAAACACATCTGGC) and OTS178 (ctgagcggccgcGAGGCATGTAGCCGCCTTGC) and restricted with BsmBI/NotI. Both restrictions were cloned into pLeo49 restricted with EcoRI/NotI.	This work (GenBank accession no. MG437038)

pTS1001	Mammalian FRB-EpoR$_{1A}$-IL-6RB$_{int}$ receptor expression vector with one alanine added C-terminally to the EpoR transmembrane helix (P$_{hCMV}$-SP-FRB-EpoR$_{1A}$-IL-6RB$_{int}$-pA) See pTS1000, second PCR was done with OTS174 (gtcacgtctcagtccgcaAATAAGCGAGACCTAATTAAAAAACACATCTGGC) and OTS178	This work (GenBank accession no. MG437039)
pTS1002	Mammalian FRB-EpoR$_{2A}$-IL-6RB$_{int}$ receptor expression vector with two alanines added C-terminally to the EpoR transmembrane helix (P$_{hCMV}$-SP-FRB-EpoR$_{2A}$-IL-6RB$_{int}$-pA) See pTS1000, second PCR was done with OTS175 (gtcacgtctcagtccgcagcaAATAAGCGAGACCTAATTAAAAAACACATCTGGC) and OTS178	This work (GenBank accession no. MG437040)
pTS1003	Mammalian FRB-EpoR$_{3A}$-IL-6RB$_{int}$ receptor expression vector with three alanines added C-terminally to the EpoR transmembrane helix (P$_{hCMV}$-SP-FRB-EpoR$_{3A}$-IL-6RB$_{int}$-pA) See pTS1000, second PCR was done with OTS176 (gtcacgtctcagtccgcagcagcAAATAAGCGAGACCTAATTAAAAAACACATCTGGC) and OTS178	This work (GenBank accession no. MG437041)
pTS1004	Mammalian FRB-EpoR$_{4A}$-IL-6RB$_{int}$ receptor expression vector with four alanines added C-terminally to the EpoR transmembrane helix (P$_{hCMV}$-SP-FRB-EpoR$_{4A}$-IL-6RB$_{int}$-pA) See pTS1000, second PCR was done with OTS177 (gtcacgtctcagtccgcagcagca gcaAATAAGCGAGACCTAATTAAAAAACACATCTGGCC) and OTS178	This work (GenBank accession no. MG437042)
pTS1005	Mammalian FKBP-EpoR$_{0A}$-IL-6RB$_{int}$ receptor expression vector (P$_{hCMV}$-SP-FKBP-EpoR$_{0A}$-IL-6RB$_{int}$-pA) See pTS1000, PCR products were cloned into pLeo50 restricted with EcoRI/NotI	This work (GenBank accession no. MG437043)
pTS1006	Mammalian FKBP-EpoR$_{1A}$-IL-6RB$_{int}$ receptor expression vector with one alanine added C-	This work

	terminally to the EpoR transmembrane helix (P_{hCMV}-SP-FKBP-$EpoR_{1A}$-IL-$6RB_{int}$-pA) See pTS1001, PCR products were cloned into pLeo50 restricted with EcoRI/NotI	(GenBank accession no. MG437044)
pTS1007	Mammalian FKBP-$EpoR_{2A}$-IL-$6RB_{int}$ receptor expression vector with two alanine added C-terminally to the EpoR transmembrane helix (P_{hCMV}-SP-FKBP-$EpoR_{2A}$-IL-$6RB_{int}$-pA) See pTS1002, PCR products were cloned into pLeo50 restricted with EcoRI/NotI	This work (GenBank accession no. MG437045)
pTS1008	Mammalian FKBP-$EpoR_{3A}$-IL-$6RB_{int}$ receptor expression vector with three alanines added C-terminally to the EpoR transmembrane helix (P_{hCMV}-SP-FKBP-$EpoR_{3A}$-IL-$6RB_{int}$-pA) See pTS1003, PCR products were cloned into pLeo50 restricted with EcoRI/NotI	This work (GenBank accession no. MG437046)
pTS1009	Mammalian FKBP-$EpoR_{4A}$-IL-$6RB_{int}$ receptor expression vector with four alanines added C-terminally to the EpoR transmembrane helix (P_{hCMV}-SP-FKBP-$EpoR_{4A}$-IL-$6RB_{int}$-pA) See pTS1003, PCR products were cloned into pLeo50 restricted with EcoRI/NotI	This work (GenBank accession no. MG437047)
pTS1012	Mammalian secreted NanoLuc expression vector (P_{hCMV}-SP-NanoLuc-pA). NanoLuc was amplified from Proinsulin-NanoLuc in pLX304 with OTS442 (ctgaactagtggtggttctggtatGGTCTTCACACTCGAAGATTTCGTTGGG) and OTS443 (ctgaggatccgctagcCGCCAGAATGCGTTCGCACAGC) restricted with SpeI/BamHI and cloned into pMM585 restricted with NheI/BamHI	This work (GenBank accession no. MG437048)

Oligonucleotides: Endonuclease cleavage sites are underlined. Annealing base pairs are indicated by capital letters.

Abbreviations: BFP, blue fluorescent protein, **cDNA**, complementary DNA, **cGST**, C-terminal glutathione S-transferase tag, **CPH1So,** sensory module of codon-optimized cyanobacterial phytochrome 1, **Elk1**, ETS domain-containing protein 1, **Env140ac**, active ribozyme from environmental samples, **EpoR**, erythropoietin receptor, **$EpoR_{0\text{-}4A}$,** EpoR extracellular and transmembrane domain with 0-4 C-terminal alanines, **$EpoR_{m}$**, modified $EpoR_{3A}$ (F93A), **$EpoR_{m0\text{-}4A}$,** modified $EpoR_{0\text{-}4A}$ (F93A), **(F93A)**, mutation of phenylalanine 93 to alanine (original numbering of full-length EpoR), **$FGFR1_{int}$,** murine fibroblast growth

factor receptor 1 intracellular domain, **FKBP**, FK506- and rapamycin-binding protein, **FRB**, FKBP-rapamycin-binding protein, **GCN4**, general control protein GCN4, **GB1,** protein G B1 domain, **gBlock**, genomic block (Integrated DNA Technologies), **hKDR**, human kinase insert domain receptor, **GEMS**, generalized extracellular molecule sensor (describes the framework PSV40-SP-antibody-EpoR$_{m}$-IL-6RB$_{m}$-pA), **IL-6RB**, IL-6 receptor subunit beta, **IL-6RB$_{int}$**, IL-6RB intracellular domain, **IL-6RB$_{m}$**, IL-6RB$_{int}$ (Y759A), **IL6-ST**, interleukin 6 signal transducer, **ITR**, inverse terminal repeat **MAPK**, mitogen-activated protein kinase, **MAPK GEMS,** describes the framework PSV40-SP-antibody-EpoR$_{m0\text{-}4A}$-FGFR1-pA, **MBP**, maltose binding protein, **MCS,** multiple cloning site, **mFGFR1**, murine fibroblast growth factor receptor 1, **NFAT**, nuclear factor of activated T-cells, **NF-κB**, nuclear factor kappa-light-chain-enhancer of activated B cells, **NLS**, nuclear localization signal, **O$_{STAT3}$**, STAT3 operator, **O$_{TetR}$**, TetR operator, **P2A**, 2A peptide, **pA**, polyadenylation signal, **P$_{hCMV}$**, cytomegalovirus immediate early promoter, **P$_{hCMVmin}$**, minimal human cytomegalovirus immediate early promoter, **P$_{hEF1a}$**, human elongation factor-1 alpha promoter, **PIP**, pristinamycin-induced protein, **PLCG**, phospholipase C gamma, **PSA**, prostate specific antigen, **PSV40**, simian virus 40 derived promoter, **P$_{T7}$**, T7 promoter, **RR120**, reactive red 120, **scFv**, single chain fragment variable, **SEAP**, secreted alkaline phosphatase, **SP**, secretory signal peptide, **spAH**, split Spinach aptamer (high binding strength), **STAT3**, signal transducer and activator of transcription 3, **SunTag$_{8\times}$**, SuperNova tag with 8 GCN4 epitope repeats, **sfGFP,** superfolder green fluorescent protein, **tGFP,** turbo green fluorescent protein, **T7$_{term}$**, T7 terminator, **TetR**, Tet repressor protein, **3'UTR**, three prime untranslated region, **5'UTR**, five prime untranslated region, **VEGFR2**, vascular endothelial growth factor receptor 2, **V$_{H}$**, variable domain heavy chain, **VHH**, variable domain of camelid heavy chain antibody, **V$_{L}$**, variable domain light chain, **VP64**, four VP16 transactivator peptides, **Y759A**, mutation of tyrosine Y759 to alanine (original numbering of full length IL-6RB)

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Supplementary Table 3: gBlock Table

gBlock name (Integrated DNA Technologies, Inc.)	**Sequence** Endonuclease cleavage sites are underlined. Coding sequences are indicated by capital letters
VHH_{A52}	caacaaactagtggatccGTGCAGTTGCAAGAATCGGGTGGTGGACTGGTTCAAGCCGGTGATAGTTTGAAACTGTCATGCGA AGCCAGTGGCGATTCCATTGGAACTTATGTCATTGGTTGGTTTCGCCAAGCACCGGGGAAAGAGCGCATTTACCTTG CCACTATTGGTCGTAACCTGGTCGGGCCGTCCGATTTTTATACACGCTACGCTGACTCTGTAAAGGGTCGCTTCGCA GTGAGTCGTGACAACGCCAAGAACACTGTAAACTTACAGATGAACTCTCTTAAACCTGAAGATACTGCTGTTTACTA TTGTGCTGCGAAAACGACAACGTGGGGGGGAAACGATCCTAATAACTGGAATTATTGGGGACAAGGCACTCAGGT GACCGTAtccggagaattcgcggccgccaacaaa
$scFv_{5d3d11}$	caacaaactagtggatccGACATTGTTATGACGCAGACCGCGCCATCCGTCTTTGTGACGCCCGGTGAATCCGTCAGTATCTCA TGTCGTTCTTCTAAGAGTTTATTGCATAGCAATGGAAATACATATTTATACTGGTTCCTTCAACGTCCGGGCCAAAG CCCCCAGTTACTTATTTATCGTATGTCCAATCTTGCATCAGGTGTCCCCGACCGTTTCAGTGGATCTGGGTCCGGCAC GGACTTCACTCTTCGTATTTCGCGTGTAGAAGCCGAAGATGTAGGAGTCTATTACTGTATGCAACATCTGGAATACC CAGTCACCTTTGGCGCCGGTACTAAGGTGGAAATCAAGCGTGGTGGTGGAGGATCAGGTGGTGGAGGGTCTGGCGG AGGCGGTAGCGGTGGAGGTGGAAGCCAAGTACAGTTACAACAATCCGGGCCGGAGTTAGTAAAACCCGGTGCATC GGTCAAGATCAGTTGCAAGGTGTCGGGTTATGCGATTTCATCTTCCTGGATGAACTGGGTTAAACAACGTCCTGGAC ACGGCTTAGAATGGATTGGTCGTATTTACCCAGGGGATGGGGATACAAAATATAACGGGAAATTCAAGGATAAGGC CACACTTACGGTAGACAAAAGCTCCTCAACTGCTTACATGCAGCTTTCCTCGCTTACGAGCGTTGATTCGGCTGTGT ACTTTTGTGCCCGCGATGGCTACCGCTATTATTTTGATTATTGGGGGCAAGGTACTAGCGTGACGGTTAGCTCAtccgg agaattcgcggccgccaaccaa
$scFv_{5a5}$	caacaaactagtggatccGACATCGTTCTTACGCAATCTCCTCCGTCCTTGGCTGTGAGCCTGGGTCAACGTGCCACCATCTCA TGTCGTGCGAGCGAGAGTATTGATTTGTACGGATTCACATTTATGCACTGGTATCAGCAAAAGCCCGGTCAACCACC CAAAATTCTGATTTACCGCGCGTCAAATTTAGAGTCAGGCATTCCTGCGCGCTTCTCAGGTTCGGGCTCACGCACAG ACTTTACATTGACAATTAACCCAGTGGAAGCAGACGATGTTGCGACATATTATTGTCAACAGACGCACGAAGATCC ATACACATTTGGTGGCGGCACTAAATTGGAAATTAAGCGTGGTGGTGGAGGATCAGGTGGTGGAGGGTCTGGCGGA GGCGGTAGCGGTGGAGGTGGAAGCCAAGTACAGTTACAGCAGAGTGGCGCAGAGTTGGCAAAACCGGGAGCCTCG GTTAAGATGTCATGCAAGACGAGTGGATACTCATTTAGCAGTTACTGGATGCACTGGGTTAAACAACGCCCAGGGC

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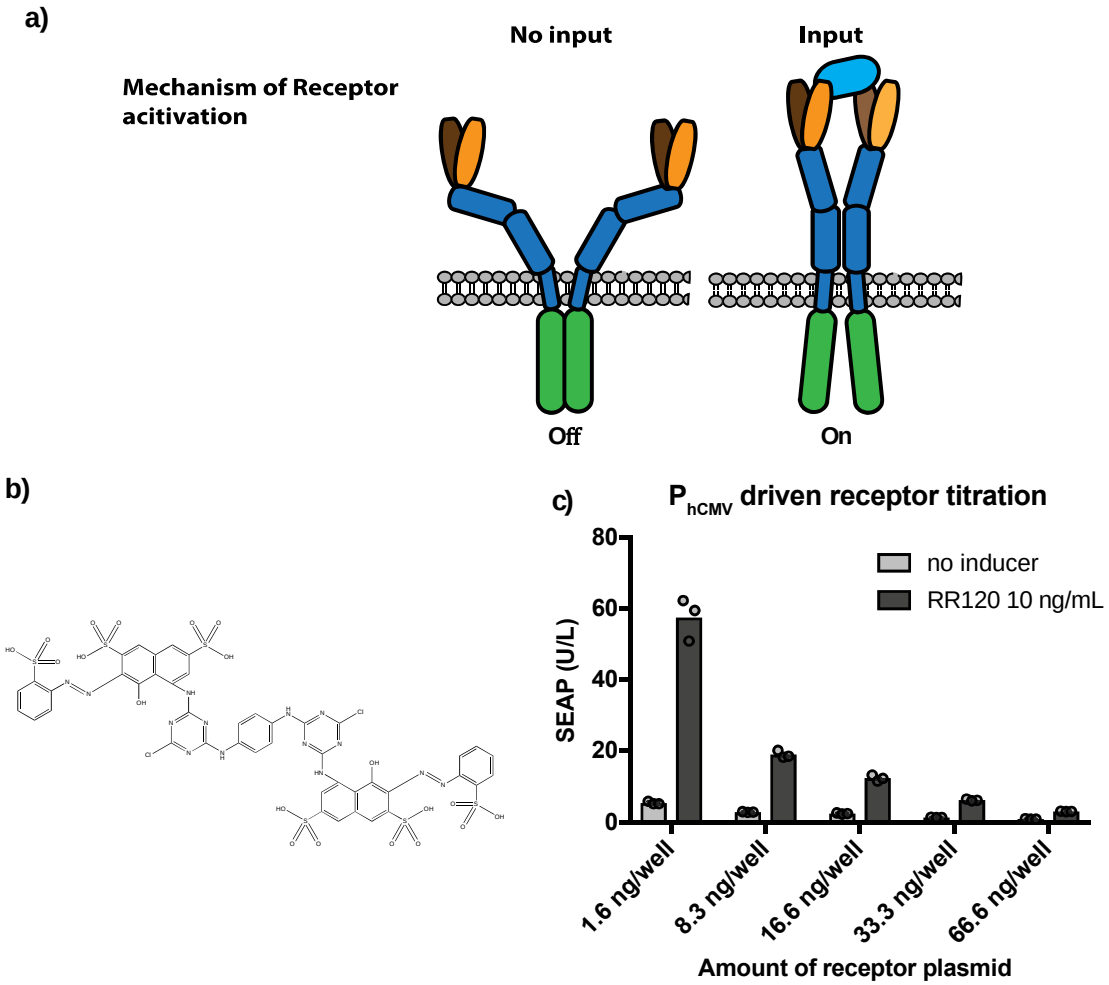
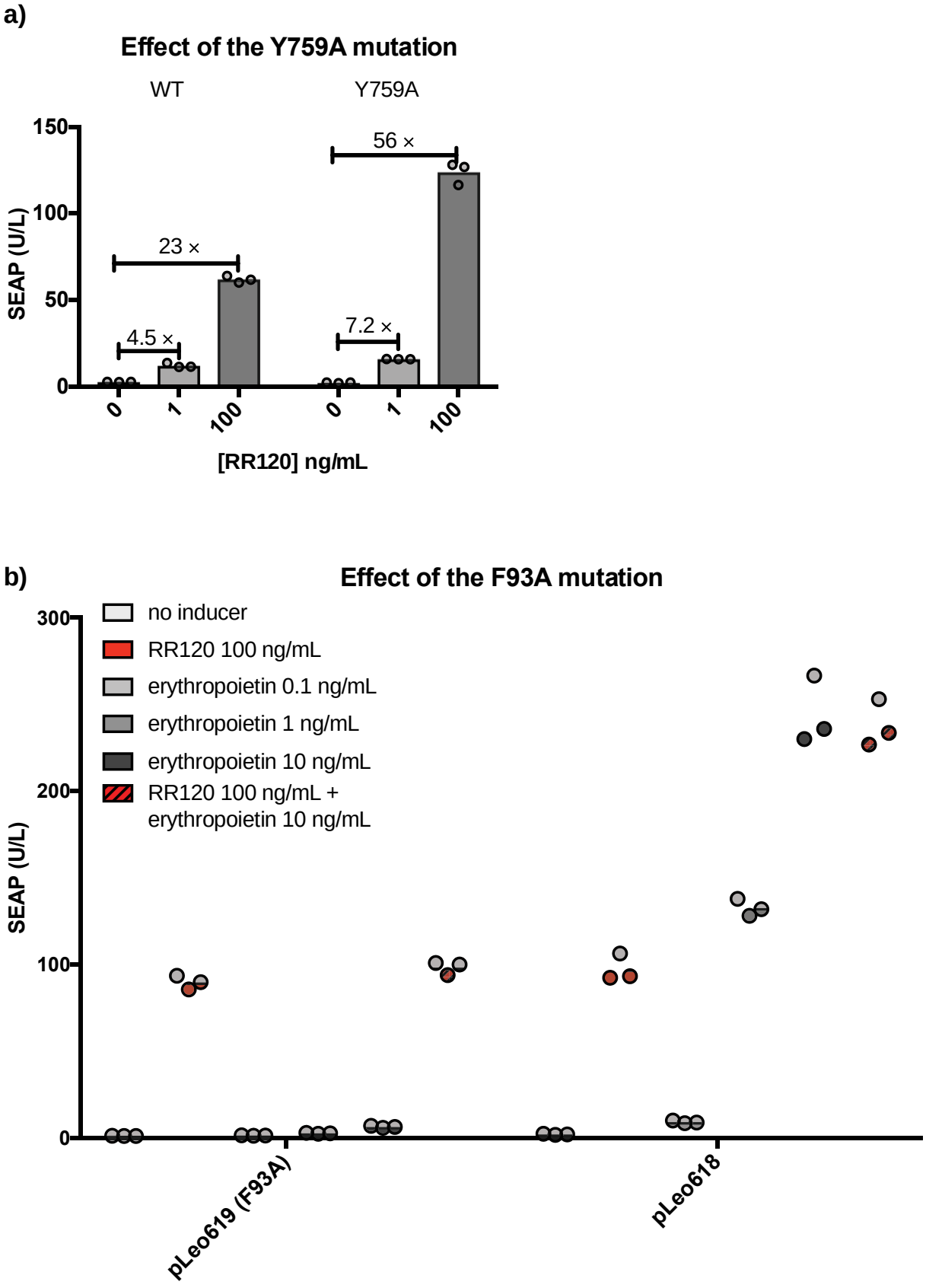
$scFv_{8g8f5}$	caacaaactagtggatccGACATTGTCCTGACCCAAAGCCCAGCGTCGTTAGCGGTGTCCCTGGGGCAACGTGCTACTATTTCC TGCAAAGCCTCCCAATCTGTTGATTTCGATGGGGACTCATACATGAACTGGTATCAGCAAAAGCCTGGACAGCCGC CTAAATTACTTATTTTCGCCGCTAGTAATCTGGCTAGTGGCATTCCTGCTCGCCTGTCAGGTTCAGGTTCGGGCACGG ATTTCACGCTGAATATCCAGCCCGTTGAGGAAGAAGATGCAGCCACATACTACTGCCAACAGTCCAATGAAGATCC CTACACTTTCGGGGGGGGCACTAAGTTGGAGATCAAGGGTGGTGGAGGATCAGGTGGTGGAGGGTCTGGCGGAGG CGGTAGCGGTGGAGGTGGAAGCCAAGTGCAGCTTCAGCAGAGCGGAGATGATTTAGTCAAGCCAGGCGCAAGTGT TAAATTATCCTGTAAAGCATCAGGTTACACATTCACTACTTATTACATCAACTGGATGCGCCAGCGCCCAGGACAAG GATTAGAGTGGATCGGTCGTATTGCTCCAGCTTCAGGCACAACTTACTCCTCAGAGATGTTCAAGGATAAGGCAAC GTTGACTGTTGATACATCTTCGAATACAGCGTACATTCAGCTTAGCTCGTTGAGTTCTGAAGATAGTGCCGTCTATTT CTGTGCTCGCGCTGATTACGGCTTTAATAGCGGGGAGGCTATGGATTATTGGGGACAAGGTACTTCTGTCACGGTGA GTTCGtccggagaattcgcggccgccaacaaa
$Nic12_{VL}$	caacaaactagtggatccCAATCCGAGTTGACCCAGCCGCCTAGTGCTAGTGGAACACCGGGACAGCGCGTGACAATTTCATG CTCCGGTTCAAGCTCAAATATCGGCTCTAATTATGTTTACTGGTATCAGCAGCTTCCAGGTACTGCGCCTAAGCTCTT AATCTACCGTAACAATCAACGTCCGTCCGGTGTGCCCGACCGCTTCTCAGGCAGTAAAAGTGGTACTTCCGCATCCC TTGCAATTTCGGGACTTCGTAGCGAAGATGAGGCAGACTATTACTGTGCAGCATGGGATGATTCCTTATCAGCTTGG GTATTCGGTGGCGGAACTCAATTAGACATTTTGGGGtccggagaattcgcggccgccaaccaa
$Nic12_{VH}$	caacaaactagtggatccCAGATGCAACTGTTGGAATCGGGACCGGGTTTGGTCAAGCCGAGCGAGACATTGAGTTTAACGTG CACGGTTTCAGGCGGATCAATTTGGGGTTGGATTCGCCAGCCGCCAGGAAAGGGACTGGAGTGGATTGGATCTATT TACTCCTCCGGTTCCACCTACTATAATCCGAGCTTGAAGTCCCGTGTGACAACAAGCGTGGATACATCCAAAAACCA GTTTTCATTGCGCCTGTCCTCAGTAACCGCAGCCGACACAGCCGTATATTATTGCGTTGCTTGGTTTGGCGACTTATT AAGTCTTAAAGGGGTAGAGCTTTGGGGCCAGGGAACTCTTGTGACGGTATCGtccggagaattcgcggccgccaaccaa

Supplementary Figures

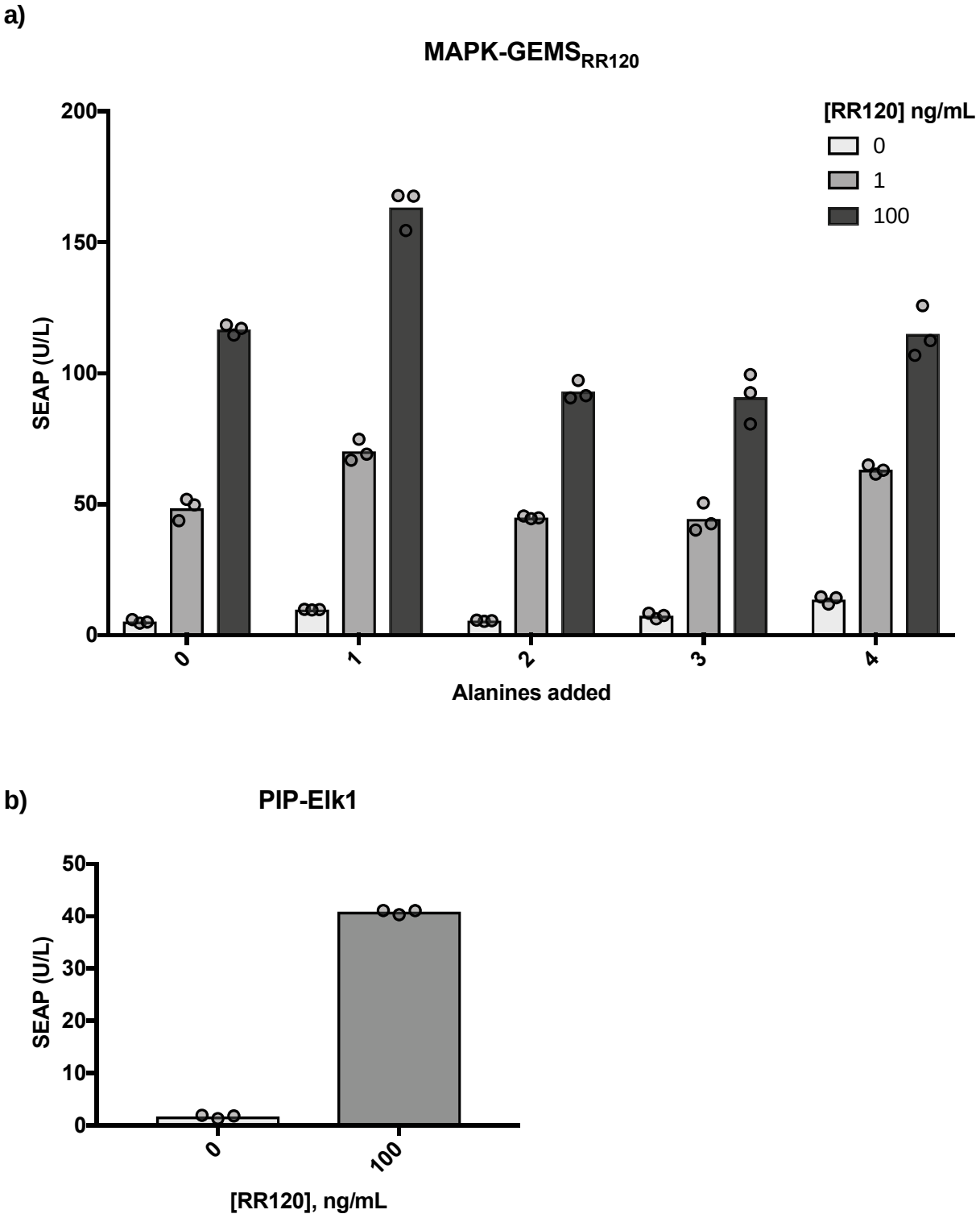


Supplementary Figure 1 | Characterization of RR120 receptors. (a) In the absence of input, the EpoR is locked in an inactive conformation. Binding of ligand facilitates interaction of intracellular domains for downstream signaling. (b) Molecular structure of RR120. VHH_{A52} binds to one half of the symmetric molecule, leading to dimerization of two antibodies. (c) RR120-induced SEAP expression increases with decreasing amount of plasmid (pLeo615) for P_{hCMV}-driven receptor expression. Graph (c) shows the mean as a bar diagram overlaid with a dot plot of individual data points of n = 3 biologically independent samples and are representative of three independent experiments.



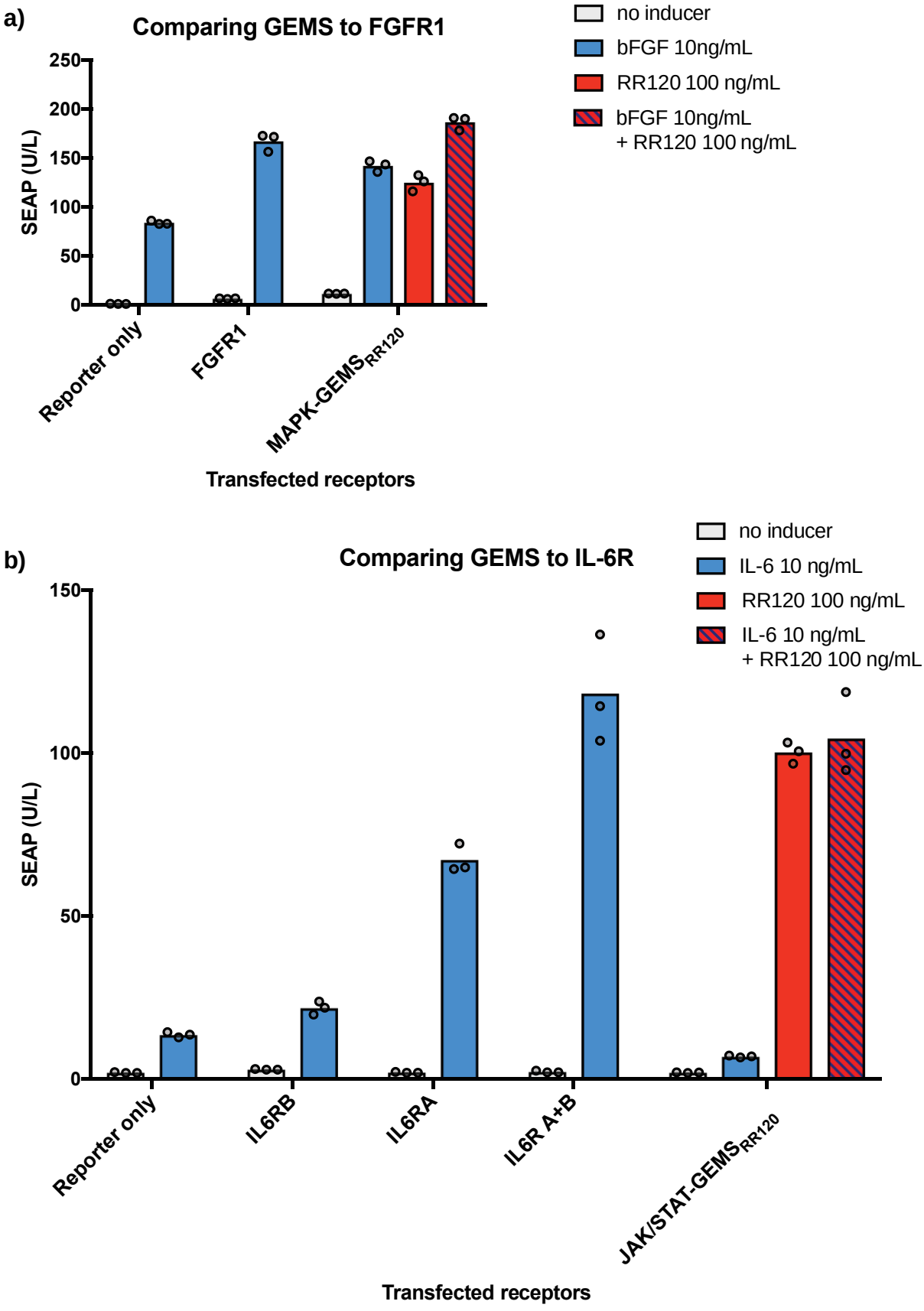
Supplementary Figure 2 | Effect of mutations in EpoR and IL6RB. (a) Introducing the mutation Y759A in the intracellular domain (pLeo618) increases the signal-to-noise ratio as well as absolute values of SEAP expression. Numbers above the bars indicate fold changes of

reporter concentration in the supernatant after 24 h induction, calculated by dividing reporter concentration in the presence of inducer by reporter concentration in the absence of inducer. (b) $GEMS_{RR120}$ (pLeo619) is induced with RR120 but not with erythropoietin. Erythropoietin also does not interfere with RR120 induced signaling. Receptors without F93A modification (pLeo618) respond strongly to erythropoietin. Graphs show the mean as a bar diagram overlaid with a dot plot of individual data points of n = 3 biologically independent samples and are representative of three independent experiments.



Supplementary Figure 3 | Scaffold optimization. (a) Adding alanines between the EpoR transmembrane domain and FGFR1 intracellular domain (pLeo628, pLeo642, pLeo643, pLeo644, pLeo645) has only minor effects on signal-to-noise ratios. The variant without alanines was chosen for further experiments. (b) A PIP-Elk1 (pAT13) and a PIP reporter (pMF199) show lower absolute expression levels but a similar dynamic range compared to the TetR-Elk1 fusion (MKp37) and the TetR reporter (pMF111), confirming the modularity of this setup. Graphs show the mean as a bar

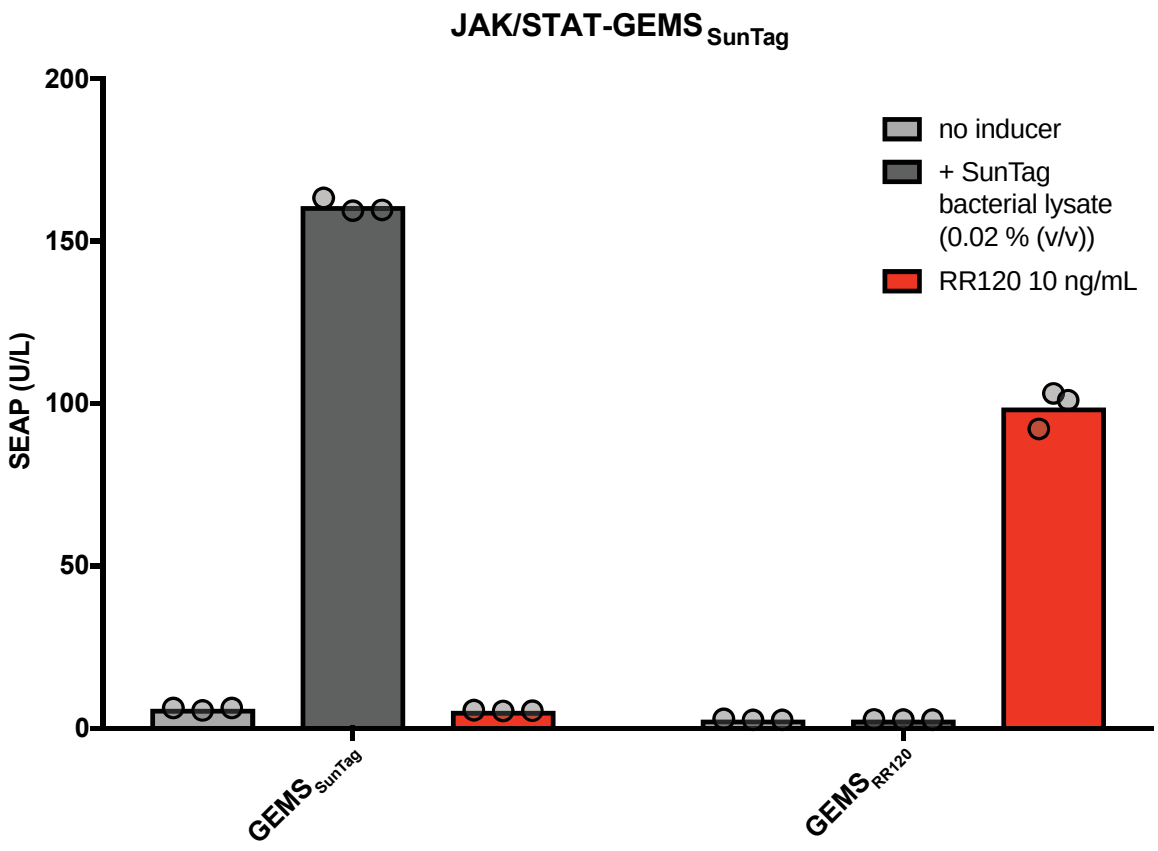
diagram overlaid with a dot plot of individual data points of n = 3 biologically independent samples and are representative of three independent experiments.



Supplementary Figure 4 | Benchmarking GEMS. (a) In HEK-293 cells, MAPK can be induced by bFGF. Overexpression of FGFR1 (pLeo698) increases reporter gene expression. (b) STAT3 can be

induced with IL-6. Overexpression of IL-6RA (pLeo696) and/or IL6-RB (pLeo697) increases reporter gene expression. Graphs show the mean as a bar diagram overlaid with a dot plot of individual data points of n = 3 biologically independent samples and are representative of three independent experiments.

a)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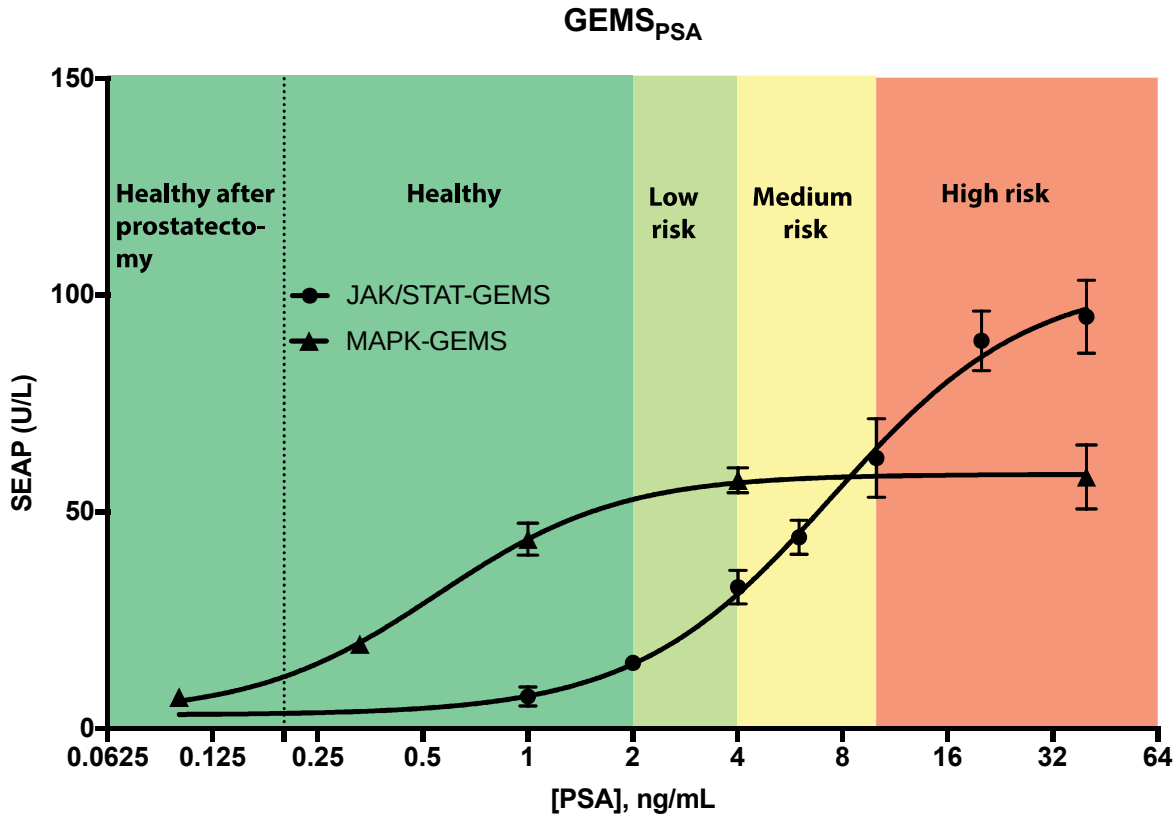
b)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MAPK-GEMS
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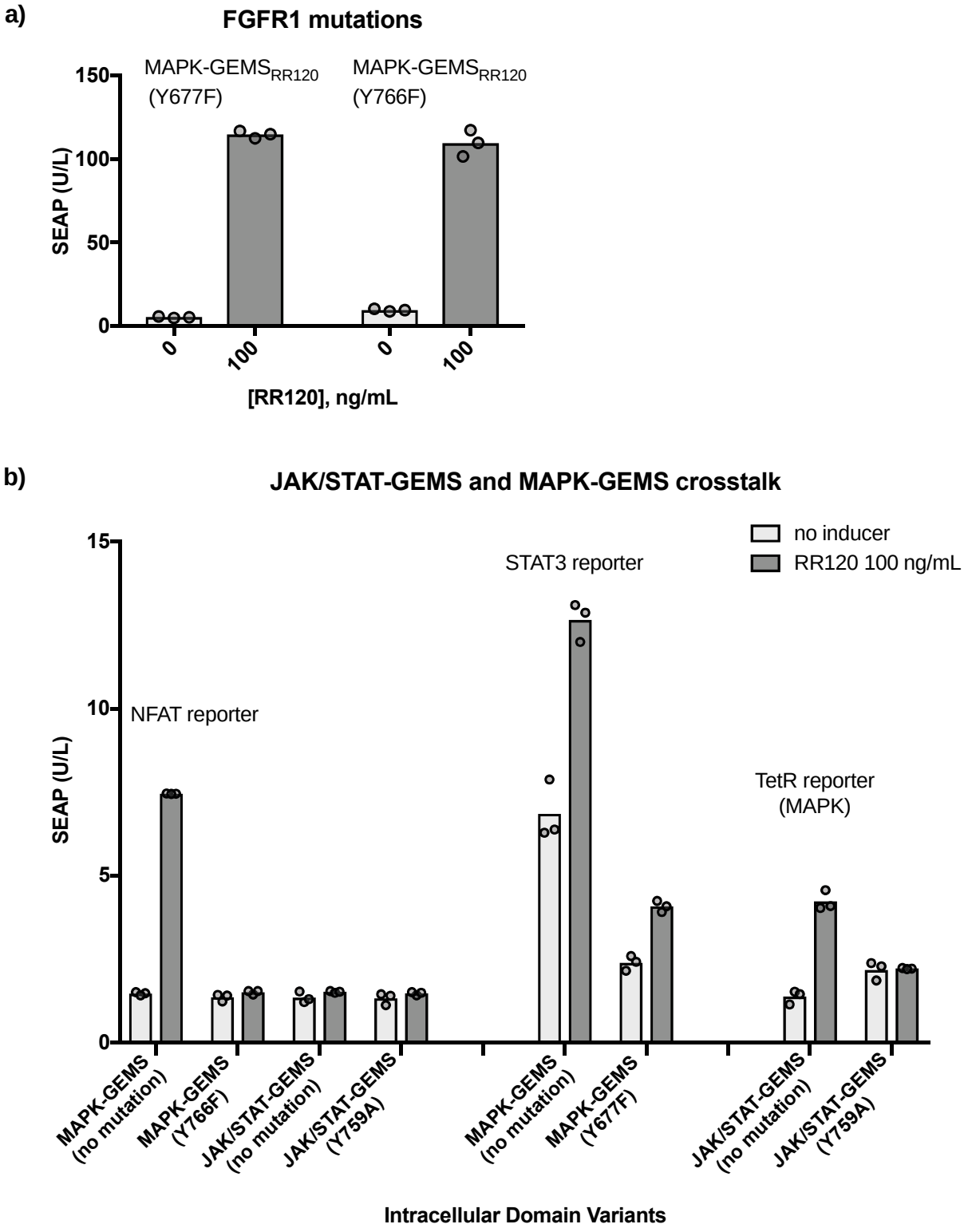
0.125
0.25
0.5
1
2
4
8
16
32
64
[PSA], ng/mL

Supplementary Figure 5 | Further analysis of $GEMS_{SunTag}$ and $GEMS_{PSA}$. (a) Bacterial crude lysate containing SunTag-mCherry induces $GEMS_{SunTag}$, but not $GEMS_{RR120}$. RR120 induces $GEMS_{RR120}$, but not $GEMS_{SunTag}$. These controls verify that reporter induction is specific to the designed inducer receptor pair. The graph shows the mean as a bar diagram overlaid with a dot plot of individual data points of n = 3 biologically independent samples and is representative of three independent experiments. (b) Dose-response curve of $GEMS_{PSA}$ presenting the same data from Figure 4 a, b. MAPK-$GEMS_{PSA}$ signal in a concentration range that is diagnostically relevant to detect biochemical recurrence after radical prostatectomy, while JAK/STAT-$GEMS_{PSA}$ exhibits an almost linear dose response over the PSA concentration range between 1 to 20 ng/mL that is critical for screening. PSA concentrations below 2 (green) are considered physiological. Concentrations between 2 to 4 ng/mL indicate a need for further monitoring (green/yellow). Concentrations between 4 to 10 ng/mL (yellow) are considered to be a diagnostic grey zone and a prostate biopsy may be recommended, depending on additional factors. Concentrations above 10 ng/mL (red) are indicative of a high risk of prostate cancer. Dose response curves were generated as four-parameter dose-response curve with GraphPad Prism 7.



Supplementary Figure 6 | Reducing GEMS crosstalk. (a) Mutations of the PLCG or STAT binding sites (PLCG:Y766F, STAT:Y677F) in the intracellular domain of FGFR1 have no observable effect on MAPK signaling. (b) MAPK-GEMS had only a minor effect on NFAT and STAT3. Mutation of the PLCG or STAT binding sites decreased the respective activation of NFAT or STAT3. JAK/STAT-GEMS without Y759A had only a minor effect on MAPK signaling. Graphs show the mean as a bar

diagram overlaid with a dot plot of individual data points of n = 3 biologically independent samples and are representative of three independent experiments.