Supplementary Information

Non-Immune Cells Equipped with T-Cell-Receptor-Like Signaling for Cancer Cell Ablation

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Inventory

Supplementary Results

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In Supplementary Table 1 and 2, the plasmids and oligonucleotides used in this study are shown. In Supplementary Figure 1-5, additional data supporting that our system worked as designed and showing detailed performance of the system are described. Please see online methods section for the protocol of each experiment.

Plasmid	Description and Cloning Strategy	Reference/Source
pHCM-CD43ex 45int-mCherry	Constitutive expression vector of CD43ex-45int-mCherry (P_{hCMV}-CD43ex-45int -pA).	James et al[1]
pLeo26	An empty plasmid having SV40 promoter (having same structure as pcDNA3.1(+)) (P_{SV40}-MCS-pA_{bGH}). SV40 promoter region was PCR amplified from pSEAP2-Control Vector (Clontech) by oDA190 and oDA191. The amplified DNA fragment was digested with *Mlu*I/*Nhe*I and inserted into corresponding site of pcDNA3.1(+).	This work
pLeo49	Constitutive Igκ leader sequence-FRB-MCS expression vector (P_{hCMV}-FRB-MCS-pA_{bGH}). FRB (bearing Igκ leader) was PCR amplified with oLeo97 and oLeo98 from pMH228[2]. The amplified fragment was digested with *Nhe*I/*Eco*RI, and was cloned into corresponding site of pcDNA3.1(+).	This work
pLeo50	Constitutive Igκ leader sequence-FKBP-MCS expression vector (P_{hCMV}-FKBP-MCS-pA_{bGH}). FKBP (bearing Igκ leader) was PCR amplified with oLeo99 and oLeo100 from pMH229[2]. The amplified fragment was digested with *Nhe*I/*Eco*RI, and was cloned into corresponding site of pcDNA3.1(+).	This work
pLeo52	Constitutive Igκ leader sequence-FRB-hIL13Rα1 expression vector (P_{hCMV}-FRB-hIL13Rα1-pA_{bGH}). hIL13Rα1 was PCR amplified from pCH023 (unpublished, provided by C. Helene, ETH-Zurich) with oLeo133 and oLeo134. The amplified fragment was digested with *Eco*RI/*Not*I, and was cloned into corresponding site of pLeo49.	This work
pLeo53	Constitutive Igκ leader sequence-FKBP-hIL4Rα expression vector (P_{hCMV}-Igκ-FKBP-hIL4Rα-pA_{bGH}). hIL4Rα was PCR amplified from pCH024 (unpublished, provided by C. Helene, ETH-Zurich) with oLeo135 and oLeo136. The amplified fragment was digested with *Eco*RI/*Not*I, and was cloned into corresponding site of pLeo50.	This work
pLeo56	Constitutive Igκ leader sequence-FKBP-mIL10Rα expression vector (P_{hCMV}-Igκ-FKBP-mIL10Rα-pA_{bGH}). mIL10Rα was cut out from pLS8[3] with *Eco*RI/*Not*I, and was inserted into corresponding site of pLeo50.	This work
pLeo57	Constitutive Igκ leader sequence-FRB-mIL10Rβ expression vector (P_{hCMV}-Igκ-FRB-mIL10Rβ-pA_{bGH}). mIL10Rβ was PCR amplified from pLS9[3] with oLeo103 and oLeo129. The amplified fragment was digested with *Eco*RV/*Not*I, and was cloned into corresponding site of pLeo49.	This work
pLS12	P_{STAT6} driven SEAP expression vector (P_{STAT6}-SEAP-pA).	Schukur et al[3]
pLS13	P_{STAT3}-driven SEAP expression vector (P_{STAT3}-SEAP-pA).	Schukur et al[3]
pLS15	Constitutive hSTAT3 expression vector (P_{hCMV}-hSTAT3-pA).	Schukur et al[3]
pLS16	Constitutive hSTAT6 expression vector (P_{hCMV}-hSTAT6-pA).	Schukur et al[3]
pRK14	Constitutive CD43ex-YFP expression vector (P_{hCMV}-CD43ex-YFP-pA_{SV40}). Extracellular and transmembrane domain of CD43 (CD43ex), whose *Nhe*I site is silently mutated, was amplified from a DNASU plasmid (HsCD00446356) by 2 step PCR. 1st PCR: By using a DNASU plasmid (HsCD00446356) as a template, One fragment was amplified by oRK4 and oRK5, and another fragment was amplified by oRK6 and oRK7. 2nd PCR: By using mixture of the 2 fragments obtained from 1st PCR as templates, the CD43ex was amplified. The amplified fragment was digested with *Nhe*I/*Age*I, and was inserted into corresponding site of pEYFP-C1.	This work
pRK17	A plasmid having a multi-cloning site followed by linker-CFP (P_{hCMV}-MCS (multiple cloning site)-CFP-pA_{bGH}). Linker-CFP was PCR amplified from pECFP-C1 (Clontech) by oRK22 and oRK23. The amplified DNA fragment was digested with *EcoRI*/*Not*I and inserted into corresponding site of pcDNA3.1(+).	This work
pRK21	Constitutive HER2-iRFP670 expression vector (P_{hCMV}-HER2-iRFP-pA_{bGH}). HER2 was PCR amplified with oRK18 (having kozak) and oRK19 by using addgene #16257 (a gift from Mien-Chie Hung[4]) as a template, and was digested with *Hin*dIII/*Not*I. iRFP670 was PCR amplified with oRK20 (having linker sequence) and oRK 21 by using pMM581 (unpublished) as a template, and was	This work

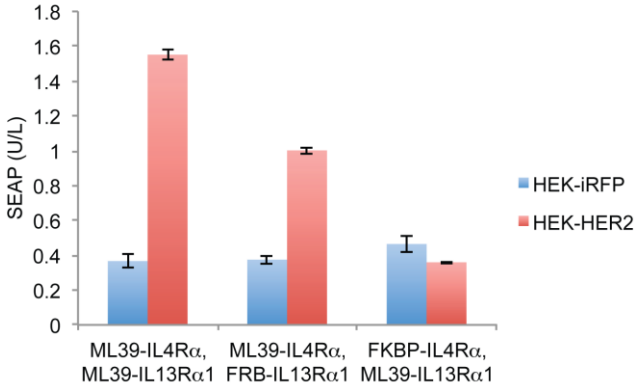
	digested with *Not*I/*Xba*I. These 2 fragments were sequentially inserted in the corresponding sites of pcDNA3.1(+).	
pRK22	Constitutive iRFP670 expression vector (P_{hCMV}-iRFP-pA_{bGH}). iRFP670 was PCR amplified with oRK30 (having kozak sequence) and oRK21 by using pMM581 (unpublished, a gift from Marius Muller, ETH Zurich) as a template, and was digested with *Hin*dIII/*Xba*I. The fragment was inserted in the corresponding site of pcDNA3.1(+).	This work
pRK96	Constitutive CD43ex-45int expression vector (P_{hCMV}-CD43ex-45int-pA_{bGH}). CD43ex-45int was cut out from pHCM-CD43TMCD45Cyt (a gift from John James and Ron Vale[1]) with *Bgl*II/*Not*I (additional treatment of the fragment with *Hin*dIII/*Xba*I was necessary to remove another fragment from the vector), and was inserted to the pcDNA 3.1 digested with *Bam*HI/*Not*I.	This work
pRK114	Constitutive Igκ leader signal-ML39-hIL13Rα1 expression vector (P_{hCMV}-ML39-hIL13Rα1-pA_{bGH}). DNA encoding ML39 (anti-HER2 scFv) (addgene #10794, a gift from Judy Lieberman[5]) was PCR amplified with oRK41 (having kozak and additional Igκ leader) and oRK139 and was digested with *Nhe*I/*Eco*RI. This fragment was inserted in the corresponding site of pLeo52.	This work
pRK115	Constitutive Igκ leader signal-ML39-full hIL4Rα expression vector (P_{hCMV} -ML39-hIL4Rα-pA_{bGH}). DNA encoding ML39 (anti HER2 scFv) (addgene #10794) was PCR amplified with oRK41 (having kozak, additional Igκ signal) and oRK139, and was digested with *Nhe*I/*Eco*RI. Also, additional fragment cut out from pLeo53 with *Eco*RI/*Xho*I (1216bp) was prepared. These 2 fragments were inserted in pLeo52 digested with *Nhe*I/*Xho*I (3 piece ligation).	This work
pRK119	Constitutive Igκ leader signal-ML39-CD28hinge-truncated hIL4Rα (trans-membrane and cytosolic domain) expression vector (P_{hCMV}-ML39-$CD28_{hin}$-hIL4Rα*Δex*-pA_{bGH}). DNA encoding transmembrane and cytosolic domain of hIL4Rα was PCR amplified by oRK143 (having additional CD28 hinge domain) and oRK140 using pLeo53 as a template and was digested with *Eco*RI/*Xba*I. This fragment was inserted in corresponding site of pRK115.	This work
pRK122	Plasmid vector expressing the same protein as pRK119 driven by SV40 promoter (P_{SV40}-ML39-hIL4Rα*Δex*-pA_{bGH}). Two fragments were prepared from pRK119 (1st fragment: 846bp digested with *Nhe*I/*Eco*RI, 2nd fragment: 3027 bp digested with *Eco*RI/*Eag*I) and were inserted into pLeo26 digested with *Nhe*I/*Eag*I (3 piece ligation)	This work
pRK123	Plasmid vector expressing the same protein as pRK114 driven by SV40 promoter (P_{SV40}-ML39-hIL13Rα1-pA_{bGH}). Protein-coding region was cut out by *Nhe*I/*Apa*I from pRK114. This fragment was inserted into corresponding site of pLeo26.	This work
pRK130	Constitutive FCU1 (Yeast cytosine deaminase (FCY1)- Yeast uracil phosphoribosyltransferase (FUR1) conjugate) expression vector (P_{hCMV}-FCU1 -pA_{bGH}). FCY1 was PCR amplified by oRK148 (having *Eco*RI-*Kpn*I-GGSGG linker-kozak sequence for constructing pRK131 as well) and oRK149 (having overlapping sequence with FUR1) from DNASU ScCD00009048. Also, FUR1 was PCR amplified with oRK150 (having overlapping sequence with FCY1) and oRK151 from DNASU ScCD00009899. Then, these 2 fragments were connected by PCR with oRK148/oRK151, yielding FCU1. This fragment was digested with *Eco*RI/*Not*I and was inserted into corresponding site of pcDNA3.1(+).	This work
pRK131	Constitutive VP22-FCU1 expression vector (P_{hCMV}-VP22-FCU1-pA_{bGH}). The DNA fragment encoding FCU1 produced while constructing pRK130 was digested with *Kpn*I/*Not*I, and was inserted into corresponding site of pVP22/myc-His (Invitrogen).	This work
pRK132	Constitutive expression vector of secreted version of VP22 targeted to HER2 by ML39. (P_{hCMV}-Igκ-ML39-FCU1-pA_{bGH}) The DNA fragment encoding FCU1 produced when constructing pRK130 was digested with *Eco*RI/*Not*I, and was inserted into pRK115 digested with *Eco*RI/*Not*I (replacement with hIL4Rα).	This work
pRK144	SV40-promoter-driven SP6 (irrelevant targeting moiety)-hIL13Rα1 expression vector (P_{SV40}-SP6-hIL13Rα1-pA_{bGH}). SP6 (including secretion signal) was PCR amplified by oRK174 and oRK177 from pAT04 (unpublished, a gift from Aizhan Tastan in ETH Zurich. This construct encodes SP6 provided by Steven A. Rosenberg[6]). The amplified DNA fragment was digested with *Nhe*I/*Eco*RI and was inserted into pRK 123 digested with *Nhe*I/*Eco*RI.	This work
pRK145	SV40-promoter-driven SP6 (irrelevant targeting moiety)-hIL4Rα*Δex* expression vector (P_{SV40}-SP6-hIL4Rα*Δex*-pA_{bGH}). The DNA fragments encoding hIL4Rα*Δex* was cut out from pRK119 with *Eco*RI/*Xba*I. This fragment was inserted in pRK144 digested with *Eco*RI/*Xba*I.	This work
pRK153	An empty plasmid having STAT6 promoter (having the same structure as	This work

	pcDNA3.1) (P_{STAT6}-MCS-pA_{bGH}). P_{STAT6} coding region was PCR amplified with oRK182 and oRK183 using pLS12 as a template. The DNA fragment was digested with *Nhe*I/*Mfe*I and was inserted into corresponding site of pcDNA3.1(+).	
pRK163	Plasmid expressing the same construct as pRK122, whose *Nhe*I site in the sequence of hIL4Rα is silently mutated. For the mutation, an one-step plasmid mutagenesis protocol[7] was used with oRK190 and oRK191 as primers, and pRK122 as a template.	This work
pRK173	Plasmid vector expressing Igκ leader sequence-9_26 (a DARPin against HER2[8])-full hIL13Rα1 under SV40 promoter (P_{SV40}-9_26-hIL13R-pA_{bGH}). The DARPin 9_26 was PCR amplified with oRK197 (having additional Igκ leader sequence) and oRK198 using 9_26_in_pQE30_2xstop_(corr31) (a gift from Pluckthun lab, ETH Zurich) as a template. This fragment was digested with *Nhe*I/*Eco*RI and was inserted into pRK123 digested with *Nhe*I/*Eco*RI.	This work
pRK174	Plasmid vector expressing Igκ leader sequence-9_26- hIL4Rα*Δex* under SV40 promoter (P_{SV40}-9_26-hIL4Rα*Δex*-pA_{bGH}). The DNA fragment coding Igκ-9_26 (the fragment produced while constructing pRK173) was digested with *Nhe*I/*Eco*RI and was inserted into corresponding site of pRK163.	This work
pRK182	Plasmid vector expressing Igκ leader sequence-Ec4 (a DARPin against Epcam[9])-hIL13Rα1 under SV40 promoter (P_{SV40}-Ec4-hIL13Rα1-pA_{bGH}). The DARPin Ec4 was PCR amplified with oRK197 (having additional Igκ leader sequence) and oRK199 using pQE30ss_Ec4_corr31 (a gift from Pluckthun lab, ETH Zurich) as a template. This fragment was digested with *Nhe*I/*Eco*RI and was inserted into pRK123 digested with *Nhe*I/*Eco*RI.	This work
pRK183	Plasmid vector expressing Igκ leader sequence-Ec4-hIL4Rα*Δex* under SV40 promoter (P_{SV40}-Ec4-hIL4Rα*Δex*-pA_{bGH}). The DNA fragment coding Igκ-Ec4 (the fragment produced when constructing pRK182) was digested with *Nhe*I/*Eco*RI and was inserted into pRK163 digested with *Nhe*I/*Eco*RI.	This work
pRK187	Lentivirus vector for constitutive expression of Epcam and ZsGreen ($P_{EF1\alpha}$-Epcam-IRES-ZsGreen). Epcam was PCR amplified with oRK200 (having additional kozak sequence) and oRK201 by using addgene #32751 (a gift from Alexander Sorkin[10]) as a template. This DNA fragment was digested with *Not*I/*Xba*I, and was inserted into corresponding site of pHIV-Luc-ZsGreen (addgene #39196, a gift from Bryan Welm) (replacement with Luc)	This work
pRK223	Plasmid vector expressing VP22-FCU1 under STAT6 promoter (P_{STAT6} -VP22-FCU1-pA_{bGH}). VP22-FCU1 was cut out from pRK131 with *Hin*dIII/*Xba*I and was inserted into pRK153 digested with *Hin*dIII/*Xba*I.	This work
pRK290	Constitutive expression vector of CD43tm-45int. (P_{hCMV}-CD43ex-45int-pA_{bGH}) The DNA fragment encoding CD43tm-45int was PCR amplified with oRK301 (bearing Igκ leader sequence and Glycine) and oRK302 by using pRK96 as a template. The DNA fragment was digested with *Nhe*I/*Apa*I, and was cloned into corresponding site of pcDNA3.1(+).	This work
pRK291	Constitutive expression vector of Lyn-CD45int (P_{hCMV}-Lyn-CD45int-pA_{bGH}) The DNA fragment encoding Lyn-CD45int was PCR amplified with oRK303 (bearing Lyn) and oRK302 by using pRK96 as a template. The DNA fragment was digested with *Nhe*I/*Apa*I, and was cloned into corresponding site of pcDNA3.1(+).	This work
pRK292	Constitutive Igκ leader signal-ML39-hIL4Rα*Δint*-CFP expression vector (P_{hCMV}-ML39-hIL4Rα*Δint*-CFP-pA_{bGH}). DNA encoding ML39-hIL4Rα*Δint* was PCR amplified by oRK304 and oRK305 using pRK115 as a template. The DNA fragment was digested with *Nhe*I/*Bst*XI, and was inserted into corresponding site of pRK17.	This work
pRK293	Constitutive Igκ leader signal-ML39-hIL4Rαtm-CFP expression vector (P_{hCMV}-ML39-hIL4Rαtm-CFP-pA_{bGH}) (tm: transmembrane domain). DNA encoding ML39-hIL4Rα*tm* was PCR amplified by oRK304 and oRK305 using pRK122 as a template. The DNA fragment was digested with *Nhe*I/*Bst*XI, and was inserted into corresponding site of pRK17.	This work
pRK295	Expression vector having the same structure as pRK293 driven by P_{SV40} promoter (P_{SV40}-ML39-hIL4Rα*Δex&int*-CFP-pA_{bGH}). Protein coding region of pRK293 was digested out with *Nhe*I/*Apa*I, and was cloned into corresponding site of pLeo26.	This work

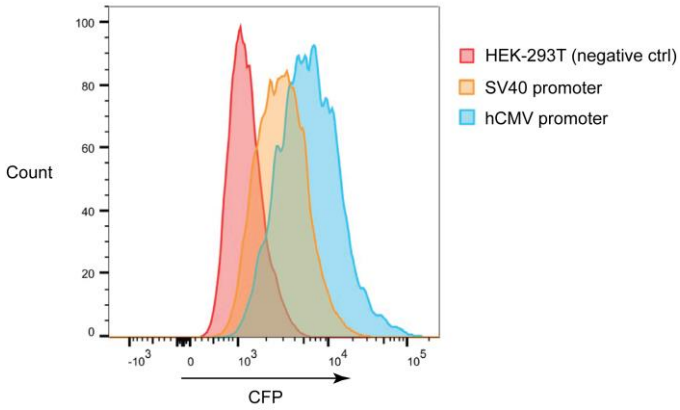
Supplementary table 1: Table of plasmids used in this study.

Oligo number	Sequence
oLeo97	AACCAAGCTAGCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCA GGTTCCACTGGTGACGGAGATATACATATGGCCTCTCGC
oLeo98	TCTAGAGAATTCTTTGCTGATACGGCGGAACAC
oLeo99	AACCAAGCTAGCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCA GGTTCCACTGGTGACATGGGCGTTCAGGTTGAAACC
oLeo100	TCTAGAGAATTCTTCCAGTTTCAGCAGTTCC
oLeo103	TTCTGCAGATATCCAATGATTCCACCCCCTGAGAAGG
oLeo129	TTCTGCAGATATCCAATGATTCCACCCCCTGAGAAGG
oLeo133	AACCAAGAATTCCCTACGGAAACTCAGCCACCT
oLeo134	AACCAAGCGGCCGCTCACTGAGAGGCTTTCTTCAGG
oLeo135	AACCAAGAATTCATGAAGGTCTTGCAGGAGCC
oLeo136	AACCAAGCGGCCGCCTAAGAGACCCTCATGTATGTGGG
oDA190	CCGATTACGCGT GATCTGCGATCTGCATCTCAATTAG
oDA191	GGCAGCTAGC GCGATTCGAAGCTTTTTGC
oRK4	ATCGgctagcGCCACCatggccacgcttctccttctccttg
oRK5	gtctccagagagctggctgccgtggt
oRK6	accacggcagccagctctctggagac
oRK7	ctgaACCGGTcctccagcgccaccagtccgccgcttctgccg
oRK18	actgAAGCTTgcCACCATGGAGCTGGCGGCCTTG
oRK19	ATTAgcggccgcCACTGGCACGTCCAGACCCAGG
oRK20	AAGTgcggccgcCGGCTCCGGAGGAATGACTAGTgcgcgtaaggtcgatctca
oRK21	ATGCtctagaCTAGAcACCGGTGGATCCGCTAGC
oRK22	atgaGAATTCggtggctccggaggaATGGTGAGCAAGGGCGAGGAGCTG
oRK23	ATTAgcggccgcCTTGTACAGCTCGTCCATGCCGAGAGT
oRK30	actgAAGCTTgcCACCATGACTAGTgcgcgtaaggtcgatctca
oRK41	taatGCTAGCgccaccATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCA GGTTCCACTGGTGACATGGCCCAGGTGCAGCTGGT
oRK139	ATTAgaattcACCTAGGACGGTCAGCTTGGTTCCTC
oRK140	ATTAtctagaCTAAGAGACCCTCATGTATGTGGGTCCCAC
oRK143	ATTTGAATTCGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTTCTAAG CCCGGATCCCTCCTGCTGGGCGTCAGCGTTTC
oRK148	attaGAATTCGGTACCggtggctcaggtggcgccaccATGGTGACAGGGGGAATGGCAAGC
oRK149	GTTCTTAAATGGTTCCGAAGACTCACCAATATCTTCAAACCAATCCTGAGGTCTTTC
oRK150	GAAGATATTGGTGAGTCTTCGGAACCATTTAAGAACGTCTACTTGCTACC
oRK151	GATTgcggccgcTTAAACACAGTAGTATCTGTCACCAAAGTCACCCAAC
oRK174	TAATgctagcgccaccATGACCCAGTCTCCAAAATTCATGTCC
oRK177	AATAgaattcCGTAGTTCCTTGGCCCCAGTAAGCAAG
oRK182	ATTAcaattgccccgaaaagtgccacctgacgtC
oRK183	actgGCTAGCttaattaaCGCGGAGGCTGGATCGGTCCCGGTGTC
oRK190	TTGGGGCcAGCAGTGGGGAAGAGGGGTATAAGCCTTTCC
oRK191	CACTGCTgGCCCCAAACCCACATTTCTCTGGGGACACAGC
oRK197	attgGCTAGCTTAATTAAgccaccATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCT GGGTTCCAGGTTCCACTGGTGACAGAGGATCGCATCACCATCACCATCACG
oRK198	ATTAgaattcATTAAGCTTTTGCAGGATTTCAGCCAGGTCC
oRK199	ATTAgaattcATTAAGCTTCGCCGCTTTTTGCAGCAC
oRK200	ATTAgcggccgcGAATTCgccaccATGGCGCCCCCGCAGGTCCTC
oRK201	attaTCTAGActaTGCATTGAGTTCCCTATGCATCTCACCCATC
oRK301	ACTTgctagccaccATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGG TTCCACTGGTGACGGAggcatgctgccagtggctgtgcttg
oRK302	TAATgggccctcacgaaccttgatttaaagctggacttgcagg
oRK303	AATgctagccaccatgggatgtataaaatcaaaagggaaagacagcgcgggaggctcaggtggctcaggaggagatcctgatgaacagcagg agcttgttg
oRK304	taatGCTAGCgccaccATGGAGACAGACACACTCCTGCTATGGG
oRK305	taatCCACCACACTGGcAATCTTGGTGATGCTGACATAGCACAACAGGC

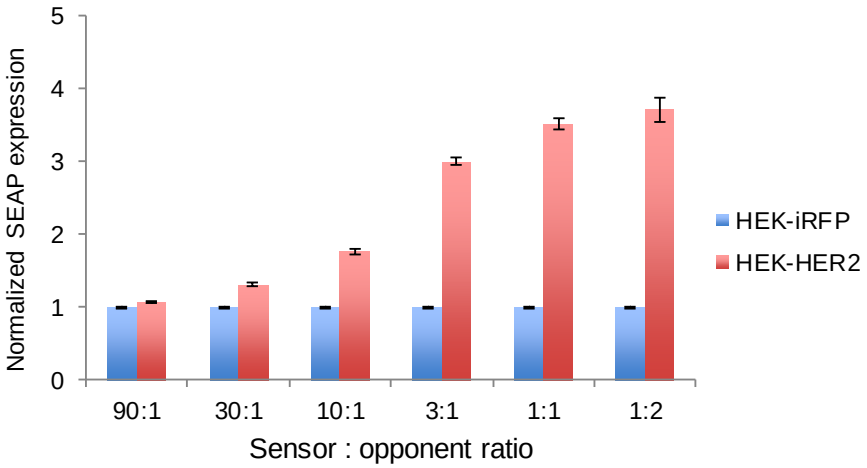
Supplementary table 2: Table of oligonucleotides used in this study.



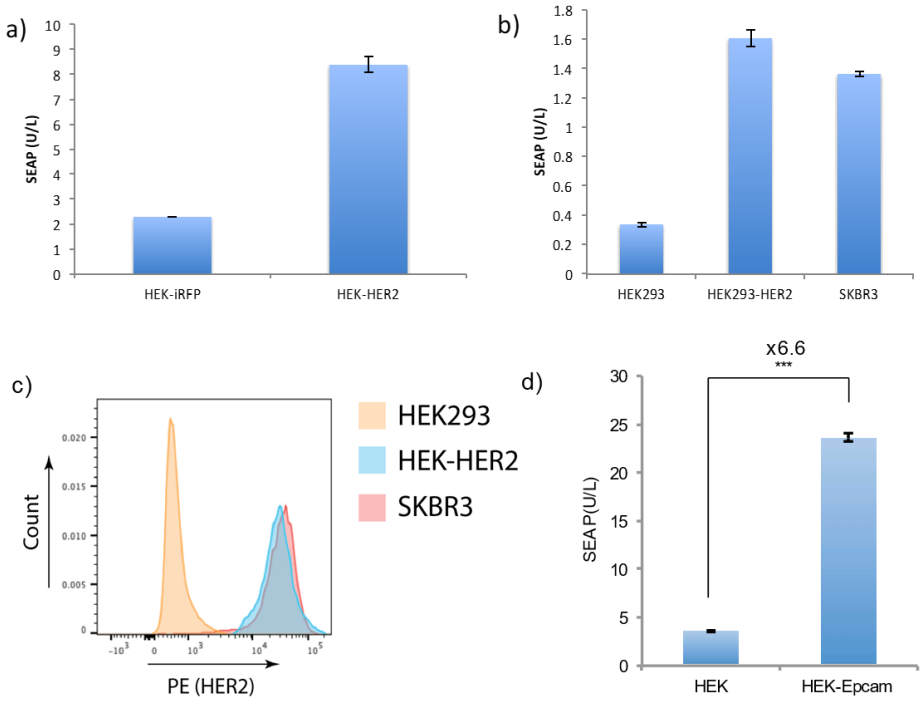
Supplementary Fig. 1. Evaluation of hetero type receptor sets. The sensor HEK-293T cells (per well of 24-well plate) were transfected with 100 ng of pRK96 (P_{hCMV}-CD43ex-45int-pA), 100 ng of pLS16 (P_{hCMV}-STAT6-pA), and 100 ng of pLS12 (P_{STAT6}-SEAP-pA), as well as 100 ng each of following interleukin receptors. Left: pRK115 (P_{hCMV}-ML39-IL4Rα-pA) and pRK114 (P_{hCMV}-ML39-IL13Rα1-pA), middle: pRK115 and pLeo52 (P_{hCMV}-FRB-IL13Rα1-pA), right: pLeo53 (P_{hCMV}-FKBP-IL4Rα-pA) and pRK114. After mixing the sensor cells with HEK-HER2 (target) or HEK-iRFP (non-target) cells, SEAP secreted from the sensor cells was assayed (with the same method as for Fig. 2b). The data are the mean ± SEM of three independent experiments measured in triplicate (n=3).



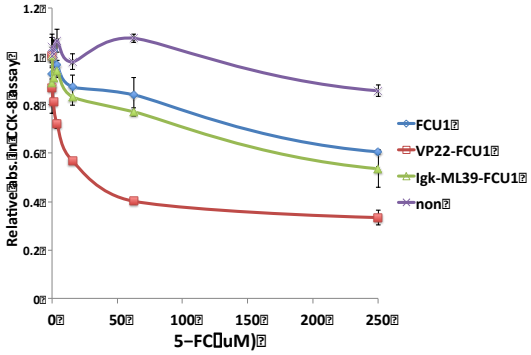
Supplementary Fig. 2. Comparison of promoter strength of hCMV promoter (P_{hCMV}) and SV40 promoter (P_{SV40}). HEK-293T cells were transfected (per well of 24-well plate) with 250 ng of either pRK293 (P_{hCMV}-ML39-IL4Rαtm-CFP-pA) (tm: transmembrane domain) or pRK295 (P_{SV40}-ML39-IL4Rαtm-CFP-pA) as well as 250 ng of pHCM-CD43ex-CD45int-mCherry (LTR-P_{hCMV}-CD43ex-45int-mCherry-LTR). Then, CFP fluorescence of mCherry positive cells was monitored by FACS BD LSRFortessa (Negative control was normal HEK-293T cells).



Supplementary Fig. 3. Effect of sensor cell / target (non-target) cell ratio on the system performance. The sensor HEK-293T cells were constructed by the same method as for Fig.3b (the optimized device with truncated receptor). These sensor cells were mixed with HEK-HER2 cells or HEK-iRFP cells under the following conditions in 1.5 mL tubes. Total number of sensor and opponent cells: 2.0×10^5 cells. Ratio of sensor cells to opponent cells: 90:1, 30:1, 10:1, 3:1, 1:1, or 1:2. Tubes were incubated at 37 °C for 30 min, then DMEM was added to make 500 µL, and the cell suspension was seeded on 24-well plates. 24 hours later, SEAP value in the supernatant was tested. (normalized to SEAP expression level when mixed with HEK-iRFP cells in each condition. The data are the mean ± SEM of three independent experiments measured in triplicate (n=3).



Supplementary Fig. 4. Generalizability of the specific cell-contact-sensing system. (a) System performance with an anti-HER2 DARPin, 9_26[8]. The sensor cells were transfected (per well of 24-well plate) with 200 ng of pRK96 (P_{hCMV}-CD43ex-45int-pA), 50 ng of pRK174 (P_{SV40}-9_26-hIL4Rα*Δex*-pA), 50 ng of pRK173 (P_{SV40} -9_26-hIL13Rα1-pA), 100 ng of pLS16 (P_{hCMV}-STAT6-pA), and 100 ng of pLS12 (P_{STAT6}-SEAP-pA). Then, the sensor cells were mixed with HEK-iRFP or HEK-HER2 cells. SEAP activity was measured at 24 hours after cell mixing (the same method as for Fig. 2b). (b) System performance in hMSC-TERT cells (against HEK-293-HER2 (model) and SKBR3 (cancer cells)). hMSC-TERT cells were transfected (per well of 24-well plate) with 200 ng of pRK96, 100 ng of pLS12, 50 ng of pRK122 (P_{SV40}-ML39-hIL4Rα*Δex*)-pA) and 50 ng of pRK123 (P_{SV40}-ML39-hIL13Rα1)-pA). Then, the transfected hMSC-TERT cells were mixed with HEK-iRFP, HEK-HER2, or SKBR3 cells. SEAP activity was measured at 24 hours after cell mixing (the same method as for Fig. 2b). (c) Confirmation of HER2 expression on HEK-HER2 cells (our model cell line) and SKBR3 cells. HEK-293, HEK-HER2, SKBR3 cells were stained with a PE-labeled anti-HER2 antibody (Biolegend #324405) in FACS buffer (DPBS containing 0.5 % BSA), and were analyzed by FACS BD LSRForsetta. HER2 expression level on SKBR3 cells is comparable to that on our model target cells, HEK-HER2. (d) Performance of Epcam-sensing device. Sensor HEK-293T cells were transfected (per well of 24-well plate) with Epcam-sensing components as follows. 50 ng of RK182 (P_{SV40}-Ec4-hIL4Rα*Δex*-pA) (Ec4: anti-Epcam DARPin[9]), 50 ng of pRK183 (P_{SV40}-Ec4-hIL13Rα1-pA), 200 ng of pRK96, 100 ng of pLS16, and 100 ng of pLS12. Then, the sensor cells were mixed with HEK-293 or HEK-Epcam cells (with the same cell number and dilution as for Fig. 2b). SEAP secreted from the sensor cells was assayed.



Supplementary Fig. 5. Evaluation of bystander effect of VP22-FCU1 for cell-based enzyme-prodrug cancer therapy in our setting. hMSC-TERT cells were transfected (per well of 24-well plate) with 50 ng of pRK122 (P_{SV40}-ML39-hIL4Rα*Δex*-pA), 50 ng of pRK123 (P_{SV40}-ML39-hIL13Rα1-pA), 200 ng of pRK96 (P_{hCMV}-CD43ex-45int-pA), 100 ng of pLS16 (P_{hCMV}-STAT6-pA), together with 100 ng of one of following plasmids: pcDNA3.1(+) (for non), pRK130 (P_{hCMV}-FCU1-pA, for expressing cytosolic FCU1), pRK 131 (P_{hCMV}-VP22-FCU1-pA, for expressing cell penetrating FCU1), or pRK132 (P_{CMV}-Igκ secretion signal (leader sequence from mouse immunoglobulin κ light chain)-ML39-FCU1-pA, for expressing secreted and HER2-targeted FCU1). At 14 hours after transfection, the medium was changed to fresh DMEM. At 24 hours after transfection, cells were detached in cell dissociation buffer, spun down, and suspended in 40 µL of DMEM. Then, 10 µL of the cell suspension (containing $6.0x10^4$ cells) was mixed with another cell suspension (40 µL) containing $2.4\ x10^5$ cells of SKBR3 cells in 1.5 mL tubes. After incubation for 30 min at 37 °C, fresh DMEM was added so that $2.5x10^3$ cells of the transfected hMSC-TERT cells and $1.0x10^4$ cells of SKBR3 cells were suspended in 100 µL, and the cell suspension was seeded on a flat bottom 96-well plate (Thermo Fischer Scientific) (100 µL/well). At 5 hrs after cell seeding, various amounts of 5-FC were added. After 4 days, cell viability was determined with a CCK-8 assay (Dojindo) according to the manufacturer's protocol. The data are the mean ± SEM of three independent experiments measured in triplicate (n=3)

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