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SUPPLEMENTARY INFORMATION

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# Dose-dependent activation of gene expression is achieved using CRISPR and small molecules that recruit endogenous chromatin machinery

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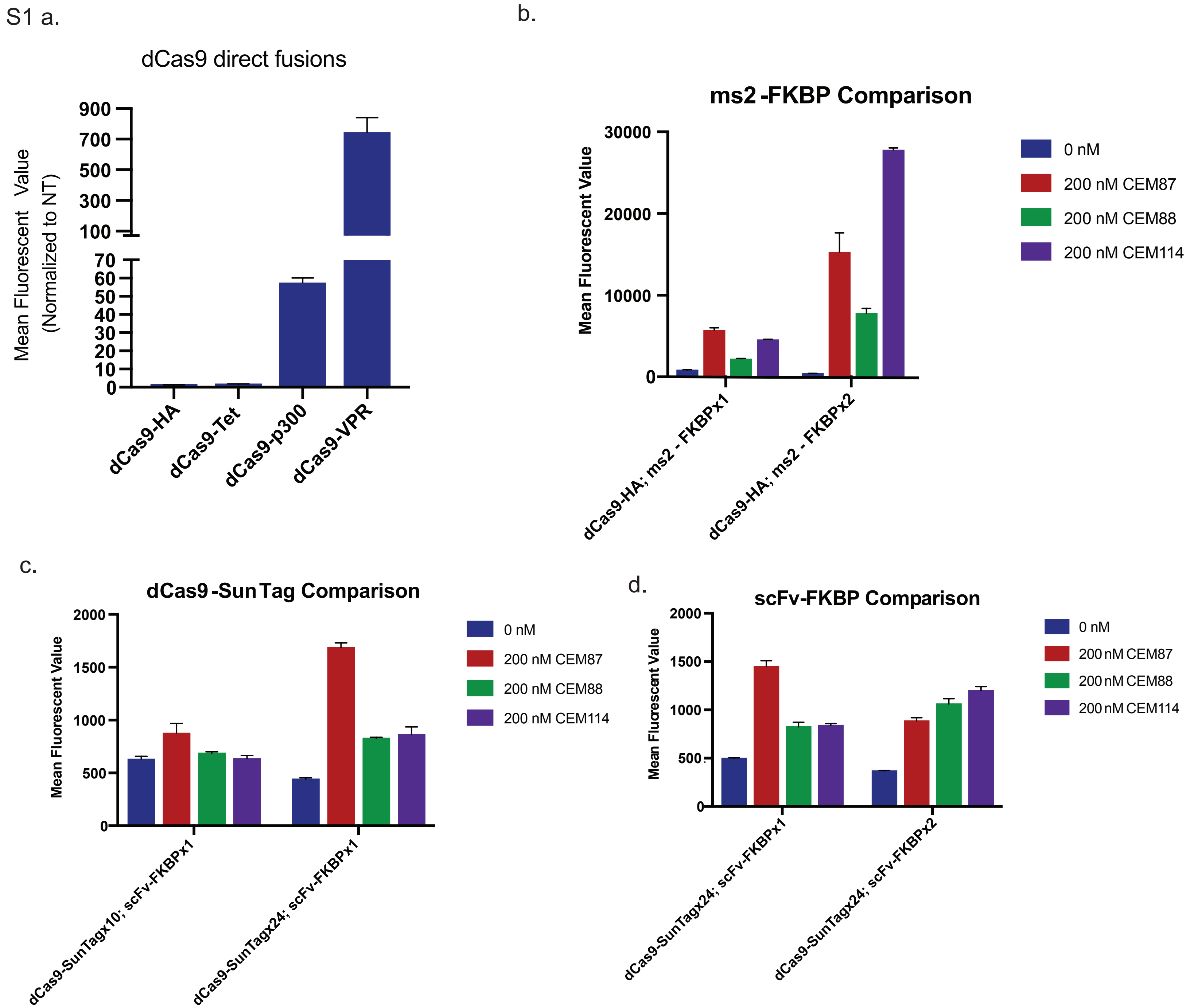
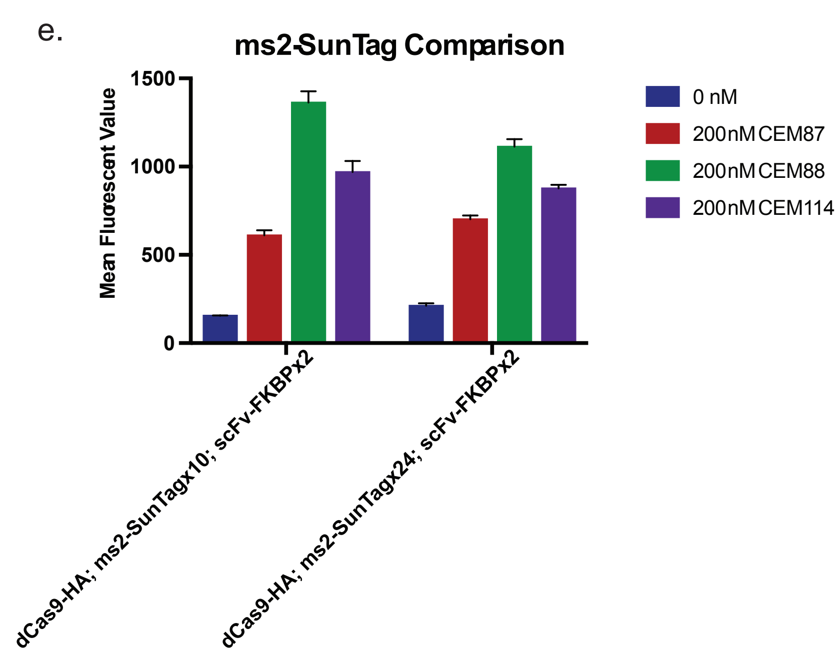
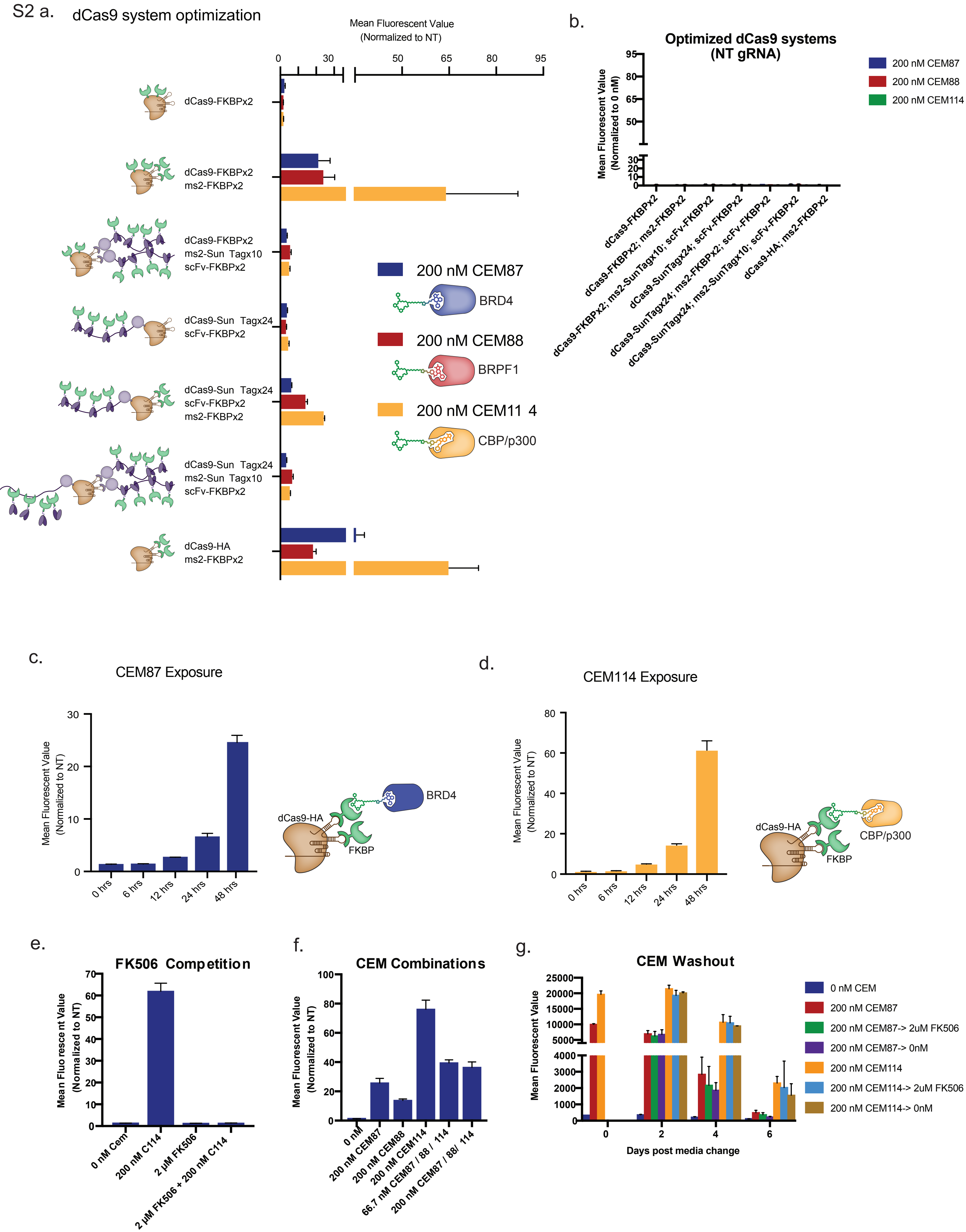


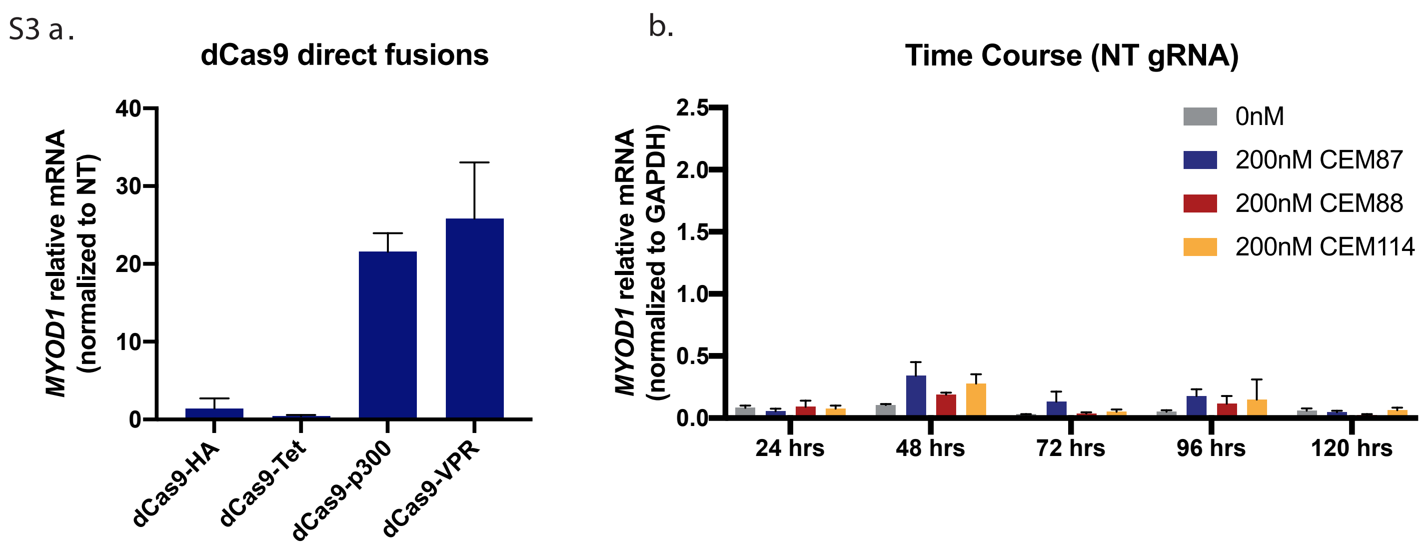



**Supplementary Fig. 1. Comparison and Optimization of dCas9 recruitment strategies. a,** HEK 293T cells were transfected with a dCas9-fusion, GFP reporter, and targeting or non-targeting gRNA. Flow cytometry was performed to compare the level of activation achieved with each protein fusion. Mean fluorescent value was normalized to NT gRNA. **b,** Cells were transfected with a GFP reporter, targeting gRNA, dCas9-HA, and one of two ms2-FKBP fusions. After 48 hours of CEM treatment, flow cytometry was performed. **c,** Cells were transfected with a GFP reporter, targeting gRNA, scFv-FKBPx1, and dCas9-SunTagx10 or dCas9-SunTagx24. After 48 hours of CEM treatment, flow cytometry was performed. **d,** Cells were transfected with a GFP reporter, targeting gRNA, dCas9-SunTagx24, and scFv-FKBPx1 or scFv-FKBPx2. After 48 hours of CEM treatment, flow cytometry was performed. **e,** Cells were transfected with a GFP reporter, targeting gRNA, dCas9-HA, scFv-FKBPx2, and ms2-SunTagx10 or ms2-SunTagx24. After 48 hours of CEM treatment, flow cytometry was performed. **a-e,** Significance was determined with three different samples, using a two-tailed student's t-test, fold change and significance reported in Supplementary Table 1. Error bars represent the standard deviation.

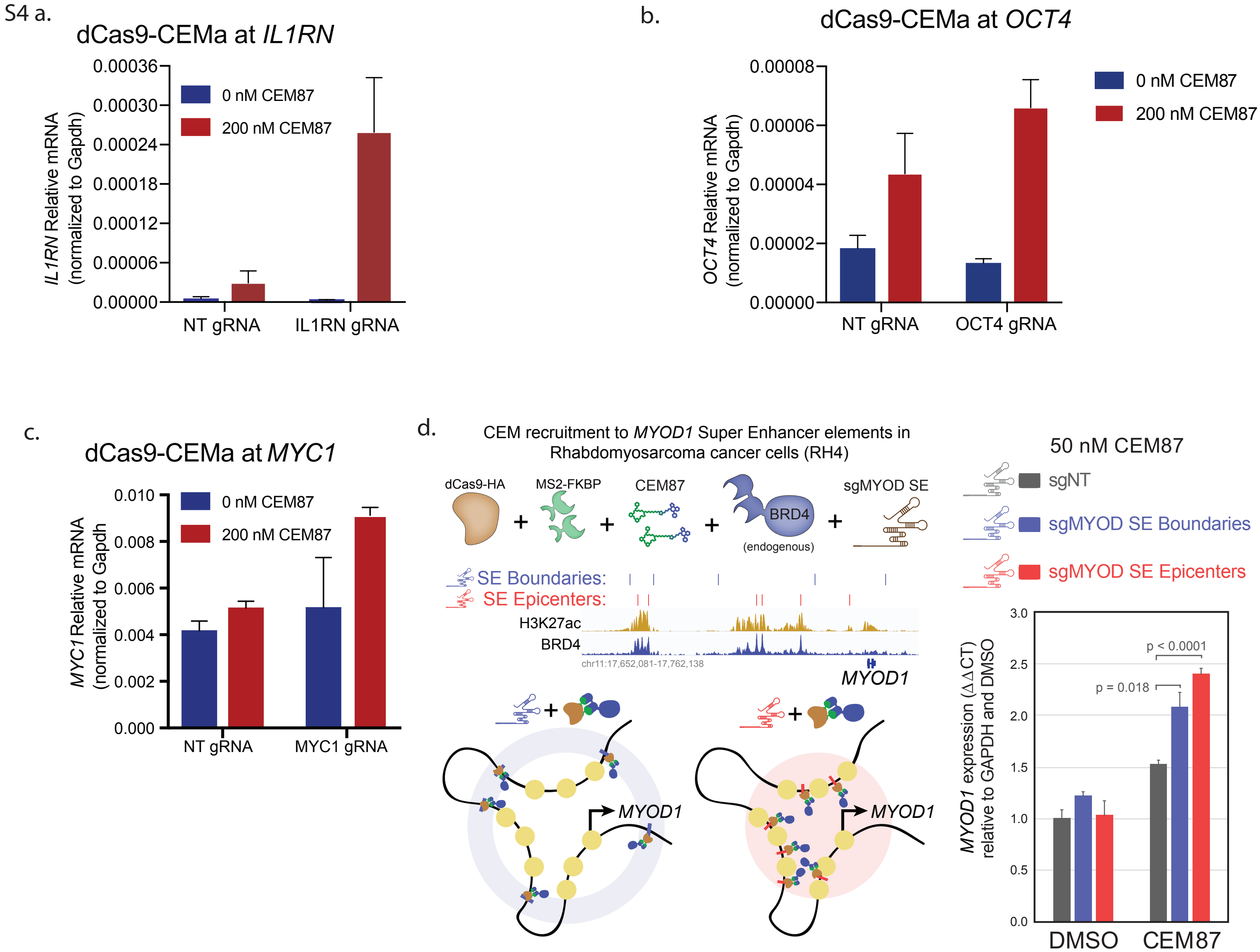


S2 a.
dCas9 system optimization
Mean Fluorescent Value
(Normalized to NT)
0
30
50
65
80
95
dCas9-FKBPx2
dCas9-FKBPx2
ms2-FKBPx2
dCas9-FKBPx2
ms2-Sun Tagx10
scFv-FKBPx2
dCas9-Sun Tagx24
scFv-FKBPx2
dCas9-Sun Tagx24
scFv-FKBPx2
ms2-FKBPx2
dCas9-Sun Tagx24
ms2-Sun Tagx10
scFv-FKBPx2
dCas9-HA
ms2-FKBPx2
200 nM CEM87
BRD4
200 nM CEM88
BRPF1
200 nM CEM11 4
CBP/p300
b.
Optimized dCas9 systems
(NT gRNA)
Mean Fluorescent Value
(Normalized to 0 nM)
95
80
65
50
30
20
10
0
200 nM CEM87
200 nM CEM88
200 nM CEM114
dCas9-FKBPx2
dCas9-FKBPx2; ms2-FKBPx2
dCas9-FKBPx2; ms2-SunTagx10; scFv-FKBPx2
dCas9-SunTagx24; scFv-FKBPx2
dCas9-SunTagx24; ms2-FKBPx2; scFv-FKBPx2
dCas9-SunTagx24; ms2-SunTagx10; scFv-FKBPx2
dCas9-HA; ms2-FKBPx2
c.
CEM87 Exposure
Mean Fluorescent Value
(Normalized to NT)
30
20
10
0
0 hrs
6 hrs
12 hrs
24 hrs
48 hrs
dCas9-HA
BRD4
FKBP
d.
CEM114 Exposure
Mean Fluorescent Value
(Normalized to NT)
80
60
40
20
0
0 hrs
6 hrs
12 hrs
24 hrs
48 hrs
dCas9-HA
CBP/p300
FKBP
e.
FK506 Competition
Mean Fluo rescent Value
(Normalized to NT)
70
60
50
40
30
20
10
0
0 nM Cem
200 nM C114
2 μM FK506
2 μM FK506 + 200 nM C114
f.
CEM Combinations
Mean Fluorescent Value
(Normalized to NT)
100
80
60
40
20
0
0 nM
200 nM CEM87
200 nM CEM88
200 nM CEM114
66.7 nM CEM87 / 88 / 114
200 nM CEM87 / 88/ 114
g.
CEM Washout
Mean Fluorescent Value
25000
20000
15000
10000
5000
4000
3000
2000
1000
0
0
2
4
6
Days post media change
0 nM CEM
200 nM CEM87
200 nM CEM87-> 2uM FK506
200 nM CEM87-> 0nM
200 nM CEM114
200 nM CEM114-> 2uM FK506
200 nM CEM114-> 0nM

**Supplementary Fig. 2. Optimization and characterization of dCas9 recruitment strategies. a,** To directly compare the various combinations of dCas9-mediated CEM recruitment, we transfected equimolar amounts of the different dCas9 components, and compared the normalized level of activation induced by CEM87, CEM88, and CEM114. Mean fluorescent values were normalized to untreated cells with identical transfection and growth conditions. **b,** Cells were transfected with equimolar amounts of GFP reporter, non-targeting gRNA, scFv-FKBPx2, a dCas9-fusion, an ms2-fusion and/or "empty" control DNA. After 48 hours of CEM treatment, flow cytometry was performed. **c,** A time course of 200 nM CEM87 was performed with CEM exposure ranging from 6 to 48 hours. Mean fluorescent value was normalized to cells expressing non-targeting gRNA. Compared to untreated cells, expression significantly increased after 12 hours. **d,** A time course of 200 nM CEM114 was performed with CEM exposure ranging from 6 to 48 hours. Mean fluorescent value was normalized to cells expressing non-targeting gRNA. Compared to untreated cells, expression significantly increased after 6 hours. **e,** Cells were transfected with dCas9-HA, ms2-FKBPx2, and targeting or non-targeting gRNA. After 48 hours of treatment with CEM114 and/or excess FK506, flow cytometry was performed. **f,** Cells were transfected with dCas9-HA, ms2-FKBPx2, and targeting or non-targeting gRNA. After transfection, cells were treated with each individual CEM, with equimolar amounts of all three CEMs at 66.7 nM each, or with equimolar amounts of all three CEMs at 200 nM each. **g,** Cells were transfected with dCas9-HA, ms2-FKBPx2, and targeting gRNA. Cells were treated with CEM87 or CEM114 for 48 hours, and flow cytometry was performed (day 0) on a subset of cells. After the initial treatment, cells had continuous CEM treatment, washout with untreated media, or washout with excess FK506. Flow cytometry was performed every 48 hours for 6 days. **a-g,** Significance was determined with three different samples, using a two-tailed student's t-test, fold change and significance reported in Supplementary Table 1. Error bars represent the standard deviation.



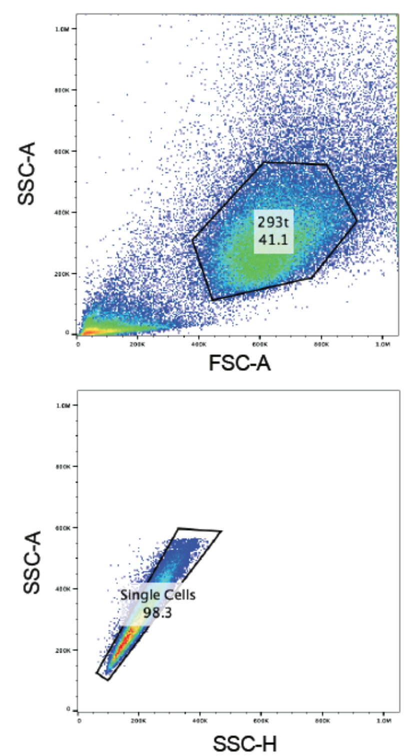

**Supplemental Fig. 3. Targeting the dCas9-CEMa system to endogenous mammalian genes. a,** Cells were infected with a dCas9-fusion (dCas9-HA, dCas9-Tet, dCas9-p300, or dCas9-VPR) and transfected with targeting gRNA (to *MYOD1*) or non-targeting gRNA. RNA was isolated 48 hours after a post-transfection media change. **b,** Cells were infected with dCas9-HA and ms2-FKBPx2 and transfected with non-targeting gRNA. Every 24 hours after CEM-treatment, RNA was isolated and relative mRNA levels were quantified. **a-b,** Significance was determined with three different samples, using a two-tailed student's t-test, fold change and significance reported in Supplementary Table 1. Error bars represent the standard deviation.




e.

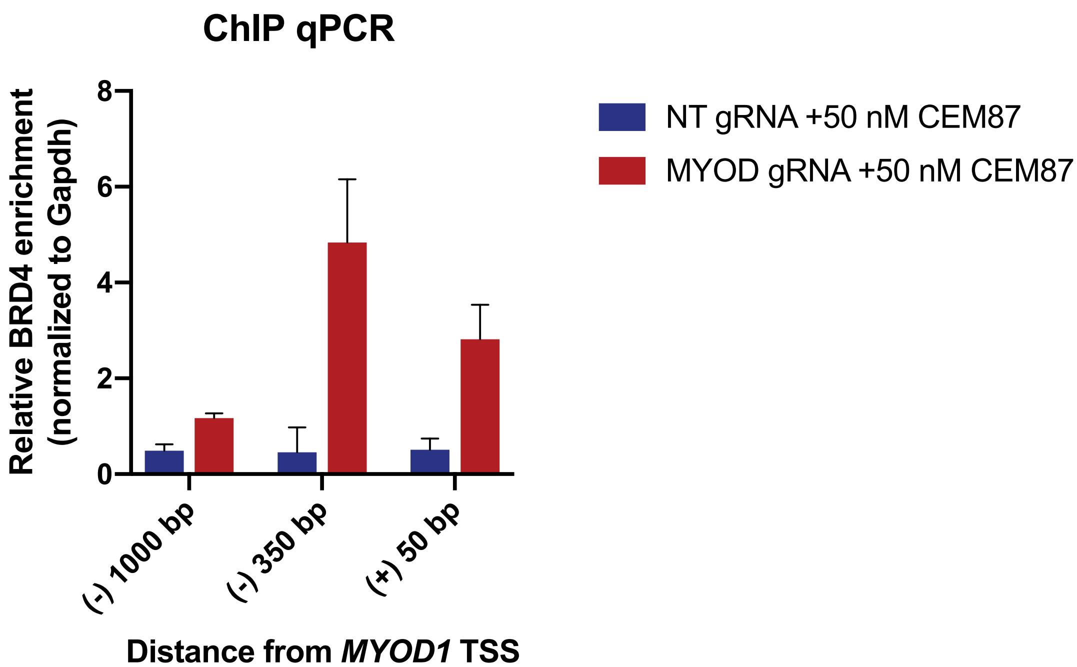
| qRT-PCR Primers | | | |
|---|---|---|---|
| Gene name: | Forward: | Reverse: | Source: |
| CXCR4 | ACTACACCGAGGAAATGGGCT | CCCACAATGCCAGTTAAGAAGA | doi: 10.1038/s41467-017-00644-y |
| IL1RN | GGAATCCATGGAGGGAAGAT | TGTTCTCGCTCAGGTCAGTG | doi: 10.1038/nbt.3199 |
| OCT4 | CGAAAGAGAAAGCGAACCAGTATCGAGAAC | CGTTGTGCATAGTCGCTGCTTGATCGC | doi: 10.1038/nbt.3199 |
| MYC1 | AAACACAAACTTGAACAGCTAC | ATTTGAGGCAGTTTACATTATGG | doi: 10.1093/nar/gkx578 |
| MYOD1 (Fig. 2) | CTCTCTGCTCCTTTGCCACA | GTGCTCTTCGGGTTTCAGGA | doi: 10.1038/nmeth.2600 |
| MYOD1 (SF. 4d) | CCACAACGGACGACTTCTATG | GAGTGCTCTTCGGGTTTCAG | this study |
| GAPDH | CAATGACCCCTTCATTGACC | TTGATTTTGGAGGGATCTCG | doi: 10.1038/nbt.3199 |

f.




**Supplemental Fig. 4. Testing dCas9-CEMa at other endogenous human loci a,** HEK 293T cells were transfected with *IL1RN*-targeting gRNA or non-targeting gRNA and treated with 200 nM of CEM87 for 48 hours. RNA was isolated and qRT-PCR was performed. Significant levels of IL1RN activation were only observed in cells expressing the targeting gRNA. **b,** Cells were transfected with *OCT4*-targeting gRNA or non-targeting gRNA and treated with 200 nM of CEM87 for 48 hours. RNA was isolated and qRT-PCR was performed. Significant levels of *OCT4* activation were only observed in cells expressing the targeting gRNA. **c,** Cells were infected with dCas9-HA and ms2-FKBP2 and transfected with targeting gRNA (to *MYC1*) or non-targeting gRNA. After 48 hours of CEM87 treatment, RNA was isolated and relative mRNA levels were quantified. **d,** Design of guide RNAs targeting the cis regulatory elements surrounding the *MYOD1* super enhancer (SE). Guide target sites are shown overlapping with ChIP-seq of H3K27ac and BRD4 in rhabdomyosarcoma cells (RH4). Recruitment was performed either at SE boundaries or SE epicenters with a set of guides cloned into a multiplexed vector. Expression of *MYOD1* upon treatment with 50 nM of CEM87 in RH4 cells containing dCas9-HA, MS2-FKBP and either sgNT, sgSE Boundaries or sgSE Epicenters, was measured with RT-qPCR after 24 hours of treatment. Error bars represent the standard deviation of three technical replicates. **e,** qRT-PCR primers for quantifying relative RNA enrichment are shown in the table. **f,** Flow cytometry results were obtained with identical gating schemes within the same experiment. **a-d,** Significance was calculated by unpaired t-test, fold change and significance reported in Supplementary Table 1. Error bars represent the standard deviation.

S5 a.




b.

| **ChIP qPCR Primers** | | |
|---|---|---|
| | **Forward:** | **Reverse:** |
| MYOD1 -1000 bp | TGGAGTCTGCACTCAACTGG | ATTTCGCCAAGGTGTCAAAC |
| MYOD1 -350 bp | GTCCCTCTTTCACGGTCTCA | AACACCCGACTGCTGTATCC |
| MYOD +50 bp | CCGCTTTCCTTAACCACAAA | CAAAGTGCTGGCAGTCTGAA |
| GAPDH | CCGGGAGAAGCTGAGTCATG | TTTGCGGTGGAAA TGTCCTT |

**Supplemental Fig. 5. ChIP-qPCR of dCas9-CEMa system at the *MYOD1* locus. a,** ChIP was performed using a BRD4 antibody on cells that were infected with dCas9-HA and ms2, transfected with targeting (to *MYOD1*, red bar) or non-targeting gRNA (blue bar), and treated with 50nM of CEM87 for 48 hours. Following ChIP, qPCR was performed with primers spanning 350 or 1000 basepairs upstream of the transcriptional start site, or 50 basepairs downstream of the transcriptional start site. **b,** the sequences of the primers used in the ChIP-qPCR experiments. **a,** Significance was determined with three different samples, using a two-tailed student's t-test, fold change and significance reported in Supplementary Table 1. Error bars represent the standard deviation.

Supplementary Table 1

| | fold change | p value |
|---|---|---|
| **Figure 1c:** | | |
| CEM87 vs untreated (dCas9-FKBPx1) | 3.1 | 2.23E-06 |
| CEM88 vs untreated (dCas9-FKBPx1) | 1.3 | 3.06E-02 |
| CEM114 vs untreated (dCas9-FKBPx1) | 1.6 | 1.70E-04 |
| CEM87 vs untreated (dCas9-FKBPx2) | 3.9 | 5.33E-04 |
| CEM88 vs untreated (dCas9-FKBPx2) | 1.5 | 8.76E-05 |
| CEM114 vs untreated (dCas9-FKBPx2) | 2.3 | 7.12E-04 |
| **Figure 1d:** | | |
| CEM87 vs untreated (dCas9-FKBPx1) | 6.7 | 9.78E-04 |
| CEM88 vs untreated (dCas9-FKBPx1) | 2.8 | 1.29E-03 |
| CEM114 vs untreated (dCas9-FKBPx1) | 3.8 | 3.43E-03 |
| CEM87 vs untreated (dCas9-FKBPx2) | 9.5 | 1.42E-04 |
| CEM88 vs untreated (dCas9-FKBPx2) | 3.9 | 7.68E-04 |
| CEM114 vs untreated (dCas9-FKBPx2) | 5.7 | 4.69E-03 |
| **Figure 1e:** | | |
| CEM87 vs untreated (dCas9-HA) | 0.9 | 8.00E-02 |
| CEM88 vs untreated (dCas9-HA) | 0.9 | 1.61E-01 |
| CEM114 vs untreated (dCas9-HA) | 1.0 | 7.10E-01 |
| **Figure 1f:** | | |
| iBet762 vs untreated (dCas9-FKBPx1) | 0.9 | 3.91E-01 |
| FK506 vs untreated (dCas9-FKBPx1) | 0.6 | 5.13E-02 |
| CEM87 vs untreated (dCas9-FKBPx1) | 3.9 | 6.66E-03 |
| **Figure 1g:** | | |
| 6.25 nM vs untreated | 2.0 | 2.75E-03 |
| 12.5 nM vs untreated | 4.0 | 2.10E-04 |
| 25 nM vs untreated | 8.0 | 5.57E-04 |
| 50 nM vs untreated | 11.4 | 1.93E-03 |
| 100 nM vs untreated | 14.2 | 3.24E-03 |
| 200 nM vs untreated | 17.0 | 3.95E-04 |
| 400 nM vs untreated | 17.6 | 1.06E-03 |
| 800 nM vs untreated | 17.3 | 2.58E-03 |
| 1600 nM vs untreated | 14.0 | 3.81E-03 |

| | | |
|---|---|---|
| **Figure 1h:** | | |
| 6.25 nM vs untreated | 1.1 | 2.04E-03 |
| 12.5 nM vs untreated | 1.5 | 3.41E-03 |
| 25 nM vs untreated | 4.4 | 2.89E-03 |
| 50 nM vs untreated | 15.5 | 1.32E-03 |
| 100 nM vs untreated | 33.8 | 5.47E-04 |
| 200 nM vs untreated | 44.8 | 8.86E-04 |
| 400 nM vs untreated | 45.1 | 2.35E-03 |
| 800 nM vs untreated | 47.5 | 8.39E-04 |
| 1600 nM vs untreated | 41.9 | 9.27E-04 |
| **Figure 2a:** | | |
| CEM87 vs untreated (24 hours) | 2.7 | 1.70E-01 |
| CEM88 vs untreated (24 hours) | 0.9 | 8.04E-01 |
| CEM114 vs untreated (24 hours) | 1.8 | 5.94E-01 |
| CEM87 vs untreated (48 hours) | 22.7 | 6.65E-03 |
| CEM88 vs untreated (48 hours) | 6.3 | 1.07E-02 |
| CEM114 vs untreated (48 hours) | 5.8 | 7.88E-02 |
| CEM87 vs untreated (72 hours) | 14.5 | 1.18E-01 |
| CEM88 vs untreated (72 hours) | 2.1 | 6.28E-01 |
| CEM114 vs untreated (72 hours) | 2.3 | 1.37E-01 |
| CEM87 vs untreated (96 hours) | 8.4 | 5.69E-02 |
| CEM88 vs untreated (96 hours) | 2.0 | 3.03E-01 |
| CEM114 vs untreated (96 hours) | 1.0 | 9.37E-01 |
| CEM87 vs untreated (120 hours) | 1.5 | 3.50E-01 |
| CEM88 vs untreated (120 hours) | 0.8 | 8.04E-01 |
| CEM114 vs untreated (120 hours) | 0.7 | 5.94E-01 |
| **Figure 2b:** | | |
| 50 nM vs untreated (targeting) | 1.5 | 4.49E-01 |
| 100 nM vs untreated (targeting) | 9.1 | 1.29E-04 |
| 200 nM vs untreated (targeting) | 12.1 | 4.67E-02 |
| 400 nM vs untreated (targeting) | 23.2 | 5.09E-03 |
| 50 nM vs untreated (non-targeting) | 1.8 | 4.49E-01 |
| 100 nM vs untreated (non-targeting) | 1.5 | 1.47E-01 |

| | | |
|---|---|---|
| 200 nM vs untreated (non-targeting) | 0.8 | 3.52E-01 |
| 400 nM vs untreated (non-targeting) | 3.9 | 3.94E-04 |
| **Figure 2c:** | | |
| CEM87 vs untreated (non-targeting) | 1.8 | 1.40E-01 |
| CEM87 vs untreated (targeting) | 12.2 | 1.08E-02 |
| **Figure 2d:** | | |
| CEM87 vs untreated (non-targeting) | 1.0 | 3.75E-01 |
| CEM87 vs untreated (targeting) | 5.6 | 1.61E-03 |
| **Figure 2e:** | | |
| CXCR4 vs NT gRNA (dCas9-VPR) | 559.7 | 2.30E-07 |
| CXCR4 vs NT gRNA (dCas9-P300) | 38.1 | 2.77E-06 |
| CXCR4 vs NT gRNA (0 nM) | 1.8 | 6.72E-02 |
| CXCR4 vs NT gRNA (50 nM CEM 87) | 18.9 | 5.30E-07 |
| CXCR4 vs NT gRNA (200 nM CEM 87) | 27.3 | 2.35E-10 |
| CXCR4 vs NT gRNA (400 nM CEM 87) | 17.5 | 6.17E-05 |
| CXCR4 vs NT gRNA (50 nM CEM 114) | 1.3 | 5.41E-02 |
| CXCR4 vs NT gRNA (200 nM CEM 114) | 3.8 | 4.35E-03 |
| CXCR4 vs NT gRNA (400 nM CEM 114) | 3.7 | 5.00E-07 |
| **Supplementary Figure 1a:** | | |
| dCas9-p300 vs dCas9 | 45.8 | 6.61E-04 |
| dCas9-tet vs dCas9 | 1.4 | 7.16E-04 |
| dCas9-VPR vs dCas9 | 592.0 | 5.43E-03 |
| **Supplementary Figure 2b:** | | |
| CEM87 vs untreated (dCas9-HA; ms2-FKBPx1) | 6.7 | 1.25E-03 |
| CEM88 vs untreated (dCas9-HA; ms2-FKBPx1) | 2.6 | 1.92E-05 |
| CEM114 vs untreated (dCas9-HA; ms2-FKBPx1) | 5.3 | 1.88E-05 |
| CEM87 vs untreated (dCas9-HA; ms2-FKBPx2) | 37.5 | 8.21E-03 |
| CEM88 vs untreated (dCas9-HA; ms2-FKBPx2) | 19.2 | 1.84E-03 |
| CEM114 vs untreated (dCas9-HA; ms2-FKBPx2) | 68.2 | 2.42E-05 |
| **Supplementary Figure 2c:** | | |
| CEM87 vs untreated (dCas9-SunTagx10; scFv-FKBPx1) | 1.4 | 3.73E-02 |
| CEM88 vs untreated (dCas9-SunTagx10; scFv-FKBPx1) | 1.1 | 4.66E-02 |
| CEM114 vs untreated (dCas9-SunTagx10; scFv-FKBPx1) | 1.0 | 8.26E-01 |

| | | |
|---|---|---|
| CEM87 vs untreated (dCas9-SunTagx24; scFv-FKBPx1) | 3.8 | 1.84E-04 |
| CEM88 vs untreated (dCas9-SunTagx24; scFv-FKBPx1) | 1.9 | 2.68E-06 |
| CEM114 vs untreated (dCas9-SunTagx24; scFv-FKBPx1) | 1.9 | 8.92E-03 |
| **Supplementary Figure 2d:** | | |
| CEM87 vs untreated (dCas9-SunTagx24; scFv-FKBPx1) | 2.9 | 1.36E-03 |
| CEM88 vs untreated (dCas9-SunTagx24; scFv-FKBPx1) | 1.7 | 6.65E-03 |
| CEM114 vs untreated (dCas9-SunTagx24; scFv-FKBPx1) | 1.7 | 7.13E-04 |
| CEM87 vs untreated (dCas9-SunTagx24; scFv-FKBPx2) | 2.4 | 1.10E-03 |
| CEM88 vs untreated (dCas9-SunTagx24; scFv-FKBPx2) | 2.9 | 1.89E-03 |
| CEM114 vs untreated (dCas9-SunTagx24; scFv-FKBPx2) | 3.3 | 7.35E-04 |
| **Supplementary Figure 2e:** | | |
| CEM87 vs untreated (dCas9-HA; ms2-SunTagx10; scFv-FKBPx2) | 3.9 | 1.28E-03 |
| CEM88 vs untreated (dCas9-HA; ms2-SunTagx10; scFv-FKBPx2) | 8.8 | 9.05E-04 |
| CEM114 vs untreated (dCas9-HA; ms2-SunTagx10; scFv-FKBPx2) | 6.3 | 1.92E-03 |
| CEM87 vs untreated (dCas9-HA; ms2-SunTagx24; scFv-FKBPx2) | 3.3 | 3.18E-05 |
| CEM88 vs untreated (dCas9-HA; ms2-SunTagx24; scFv-FKBPx2) | 5.2 | 3.06E-04 |
| CEM114 vs untreated (dCas9-HA; ms2-SunTagx24; scFv-FKBPx2) | 4.1 | 6.37E-06 |
| **Supplementary Figure 2c:** | | |
| 6 hours vs untreated | 1.1 | 2.29E-01 |
| 12 hours vs untreated | 2.0 | 3.41E-04 |
| 24 hours vs untreated | 5.0 | 4.95E-03 |
| 48 hours vs untreated | 18.5 | 1.15E-03 |
| **Supplementary Figure 2d:** | | |
| 6 hours vs untreated | 1.2 | 1.88E-02 |
| 12 hours vs untreated | 3.8 | 1.18E-06 |
| 24 hours vs untreated | 10.8 | 7.67E-04 |
| 48 hours vs untreated | 46.2 | 1.96E-03 |
| **Supplementary Figure 2e:** | | |
| CEM114 vs untreated | 45.4 | 1.26E-03 |
| FK506 vs untreated | 0.9 | 1.07E-01 |
| FK506 + CEM114 vs untreated | 1.0 | 5.66E-01 |
| **Supplementary Figure 2f:** | | |
| CEM87 vs untreated | 18.9 | 5.51E-03 |

| | | |
|---|---|---|
| CEM88 vs untreated | 10.2 | 2.26E-03 |
| CEM114 vs untreated | 56.0 | 2.35E-03 |
| CEM87 + CEM88 + CEM114 (66.7 nM each) vs untreated | 29.1 | 9.37E-04 |
| CEM87 + CEM88 + CEM114 (200 nM each) vs untreated | 26.8 | 3.80E-03 |
| **Supplementary Figure 2g:** | | |
| CEM87 vs untreated (day 0) | 29.3 | 1.90E-04 |
| CEM114 vs untreated (day 0) | 57.7 | 1.15E-03 |
| CEM87 vs untreated (day 2) | 19.1 | 8.58E-03 |
| CEM87 -> FK506 vs untreated (day 2) | 17.3 | 1.99E-02 |
| CEM87 -> 0 nM vs untreated (day 2) | 18.7 | 1.77E-02 |
| CEM114 vs untreated (day 2) | 59.0 | 1.02E-03 |
| CEM114 -> FK506 vs untreated (day 2) | 53.2 | 2.36E-03 |
| CEM114 -> 0 nM vs untreated (day 2) | 55.3 | 1.02E-04 |
| CEM87 vs untreated (day 4) | 13.1 | 4.88E-02 |
| CEM87 -> FK506 vs untreated (day 4) | 10.0 | 9.90E-02 |
| CEM87 -> 0 nM vs untreated (day 4) | 8.6 | 4.17E-02 |
| CEM114 vs untreated (day 4) | 49.2 | 1.74E-02 |
| CEM114 -> FK506 vs untreated (day 4) | 48.2 | 1.39E-02 |
| CEM114 -> 0 nM vs untreated (day 4) | 43.7 | 5.53E-03 |
| CEM87 vs untreated (day 6) | 4.3 | 4.54E-02 |
| CEM87 -> FK506 vs untreated (day 6) | 3.3 | 6.77E-02 |
| CEM87 -> 0 nM vs untreated (day 6) | 2.1 | 1.61E-01 |
| CEM114 vs untreated (day 6) | 20.4 | 1.10E-02 |
| CEM114 -> FK506 vs untreated (day 6) | 17.9 | 1.77E-01 |
| CEM114 -> 0 nM vs untreated (day 6) | 13.8 | 4.45E-02 |
| **Supplementary Figure 3a:** | | |
| dCas9-p300 vs dCas9 | 18.9 | 4.88E-04 |
| dCas9-tet vs dCas9 | 0.3 | 6.02E-01 |
| dCas9-VPR vs dCas9 | 21.1 | 1.21E-02 |
| **Supplementary Figure 3b:** | | |
| CEM87 vs untreated (24 hours) | 0.7 | 3.23E-01 |
| CEM88 vs untreated (24 hours) | 1.1 | 7.83E-01 |
| CEM114 vs untreated (24 hours) | 0.9 | 9.39E-01 |

| | | |
|---|---|---|
| CEM87 vs untreated (48 hours) | 3.2 | 8.14E-02 |
| CEM88 vs untreated (48 hours) | 1.8 | 6.77E-03 |
| CEM114 vs untreated (48 hours) | 2.6 | 7.98E-02 |
| CEM87 vs untreated (72 hours) | 4.4 | 2.64E-01 |
| CEM88 vs untreated (72 hours) | 1.2 | 6.61E-01 |
| CEM114 vs untreated (72 hours) | 1.7 | 3.09E-01 |
| CEM87 vs untreated (96 hours) | 3.4 | 7.50E-02 |
| CEM88 vs untreated (96 hours) | 2.2 | 3.92E-01 |
| CEM114 vs untreated (96 hours) | 2.9 | 7.39E-01 |
| CEM87 vs untreated (120 hours) | 0.8 | 4.22E-01 |
| CEM88 vs untreated (120 hours) | 0.4 | 1.76E-01 |
| CEM114 vs untreated (120 hours) | 1.1 | 9.39E-01 |
| **Supplementary Figure 4a:** | | |
| CEM87 vs untreated (non-targeting) | 1.2 | 3.62E-02 |
| CEM87 vs untreated (targeting) | 1.8 | 8.21E-02 |
| **Supplementary Figure 4b:** | | |
| CEM87 vs untreated (non-targeting) | 2.4 | 7.35E-02 |
| CEM87 vs untreated (targeting) | 4.9 | 9.27E-03 |
| **Supplementary Figure 4c:** | | |
| CEM87 vs untreated (non-targeting) | 5.5 | 1.47E-01 |
| CEM87 vs untreated (targeting) | 92.5 | 3.33E-02 |
| **Supplementary Figure 4d:** | | |
| Boundary gRNA vs NT gRNA | 1.4 | 1.80E-02 |
| Epicenter gRNA vs NT gRNA | 1.6 | 1.00E-04 |
| **Supplementary Figure 5a:** | | |
| Targeting vs non-targeting (-1000 bp) | 2.4 | 2.39E-03 |
| Targeting vs non-targeting (-350 bp) | 10.7 | 1.80E-02 |
| Targeting vs non-targeting (+50 bp) | 5.5 | 2.25E-02 |

Supplementary Table 2

**Table of gRNAs and source:**

| Target: | Protospacer Sequence (5'-3') | Source |
|---|---|---|
| Tre3g x6 | TACGTTCTCTATCACTGATA | doi: 10.1038/nmeth.4042 |
| MYOD1 gRNA #1 | CCTGGGCTCCGGGGCGTTT | doi: 10.1038/nmeth.2600 |
| MYOD1 gRNA #2 | GGCCCCTGCGGCCACCCCG | doi: 10.1038/nmeth.2600 |
| MYOD1 gRNA #3 | CTCCCTCCCTGCCCGGT AG | doi: 10.1038/nmeth.2600 |
| MYOD1 gRNA #4 | AGGTTTGGAAAGGGCGTGC | doi: 10.1038/nmeth.2600 |
| CXCR4 gRNA #1 | GCAGACGCGAGGAAGGAGGGCGC | doi: 10.1038/s41467-017-00644-y |
| CXCR4 gRNA #2 | CCGACCACCCGCAAACAGCA | doi: 10.1038/s41467-017-00644-y |
| CXCR4 gRNA #3 | GCCTCTGGGAGGTCCTGTCCGGCTC | doi: 10.1038/s41467-017-00644-y |
| OCT4 gRNA #1 | ACTCCACTGCACTCCAGTCT | doi: 10.1093/nar/gku109 |
| OCT4 gRNA #2 | TCTGTGGGGGACCTGCACTG | doi: 10.1093/nar/gku109 |
| OCT4 gRNA #3 | GGGGCGCCAGTTGTGTCTCC | doi: 10.1093/nar/gku109 |
| OCT4 gRNA #4 | ACACCATTGCCACCACCATT | doi: 10.1093/nar/gku109 |
| IL1RN gRNA #1 | TGTACTCTCTGAGGTGCTC | doi: 10.1038/nmeth.2600 |
| IL1RN gRNA #2 | ACGCAGATAAGAACCAGTT | doi: 10.1038/nmeth.2600 |
| IL1RN gRNA #3 | CATCAAGTCAGCCATCAGC | doi: 10.1038/nmeth.2600 |
| IL1RN gRNA #4 | GAGTCACCCTCCTGGAAAC | doi: 10.1038/nmeth.2600 |

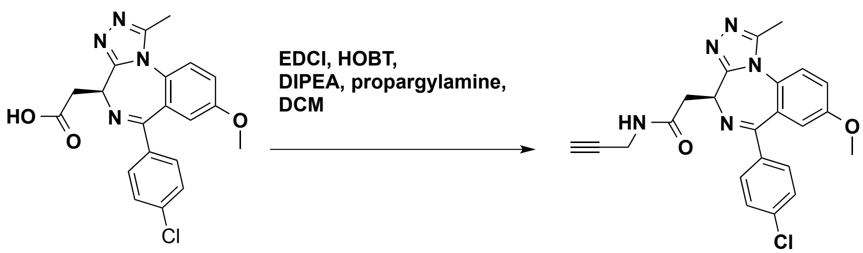
| | | |
|---|---|---|
| MYC1 gRNA #1 | GGAATGATAGAGGCATAAGG | doi: 10.1093/nar/gkx578 |
| MYC1 gRNA #2 | GTCAAACAGTACTGCTACGG | doi: 10.1093/nar/gkx578 |
| MYC1 gRNA #3 | GGGCGCTTATGGGGAGGGTG | doi: 10.1093/nar/gkx578 |
| MYC1 gRNA #4 | GGCGAGGGTCTGGACGGCTG | doi: 10.1093/nar/gkx578 |
| MYOD1 Epicenters | (1) GGGAACTTGAGCCTGCACCA (2) CAGGCAAGAACATAGATCAG (3) GGCCAGGGTTTTTTTCAGTG (4) AGATGGTCCACACTGATCTG (5) CTTCCTATAAACTTCTGAG (6) GGCTGGATTGGGTTTCCAG | this manuscript |
| MYOD1 Boundaries | (1) CTATCAGGGATGATGATCCA (2) TGAGGCAACTGAAATATGTG (3) ACTTAACAAAGATTTAAGAG (4) TCTTAGTTATGACTTTTGAG | this manuscript |
| CXCR4 multiplexed | (1) GCAGACGCGAGGAAGGAGGGCGC (2) AGCCGGACAGGACCTCCCAG (3) GACCCTGCTGTTTGCGGGTGGT (4) GAGGGAGAAGGCGGGGTGG | doi: 10.1038/s41467-017-00644-y ; doi: 10.1016/j.cell.2013.06.044 ; doi: 10.1016/j.cell.2014.11.052 |

Supplementary Table 3

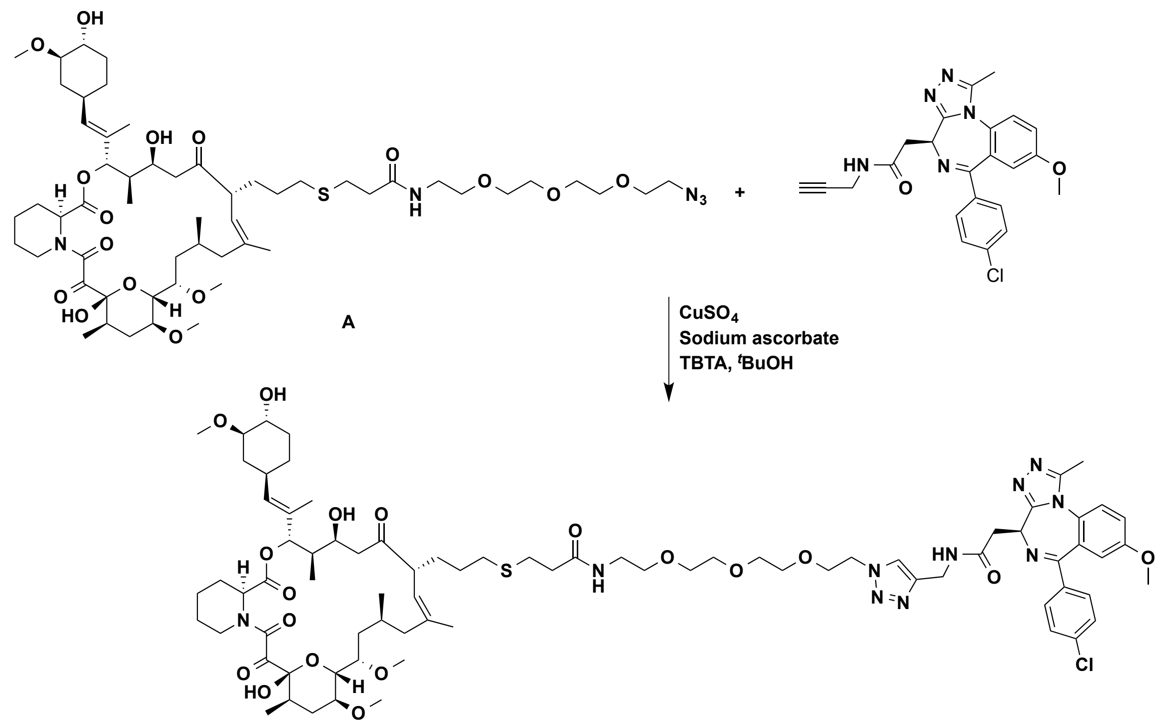
| Figure: | dCas9 (µg/well) | total gRNA (µg/well) | reporter (µg/well) | ms2-X, scFv-X and/or empty vector (µg/well) | plate format |
|---|---|---|---|---|---|
| Figure 1c | 0.75 | 0.2 | 0.05 | n/a | 12 well |
| Figure 1d | 0.75 | 0.2 | 0.05 | n/a | 12 well |
| Figure 1e | 0.75 | 0.2 | 0.05 | n/a | 12 well |
| Figure 1f | 0.75 | 0.2 | 0.05 | n/a | 12 well |
| Figure 1g | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Figure 1h | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Figure 2a | n/a | 3 | n/a | n/a | 6 well |
| Figure 2b | n/a | 3 | n/a | n/a | 6 well |
| Figure 2c | n/a | 3 | n/a | n/a | 6 well |
| Figure 2d | n/a | 3 | n/a | n/a | 6 well |
| Figure 2e | n/a | 1 | n/a | n/a | 12 well |
| Figure 3a | n/a | 36 | n/a | n/a | 15 cm |
| Figure 3b | n/a | 36 | n/a | n/a | 15 cm |
| Figure 3c | n/a | 3 | n/a | n/a | 6 well |
| Figure 3d | n/a | 3 | n/a | n/a | 6 well |
| Supplementary Figure 1a | 0.75 | 0.2 | 0.05 | n/a | 12 well |
| Supplementary Figure 1b | 0.5 | 0.15 | 0.05 | 0.3 | 12 well |
| Supplementary Figure 1c | 0.5 | 0.15 | 0.05 | 0.3 | 12 well |
| Supplementary Figure 1d | 0.5 | 0.15 | 0.05 | 0.3 | 12 well |
| Supplementary Figure 1e | 0.5 | 0.15 | 0.05 | 0.3 | 12 well |
| Supplementary Figure 2a | 0.5 | 0.15 | 0.05 | 0.3 | 12 well |
| Supplementary Figure 2b | 0.5 | 0.15 | 0.05 | 0.3 | 12 well |
| Supplementary Figure 2c | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Supplementary Figure 2d | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Supplementary Figure 2e | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Supplementary Figure 2f | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Supplementary Figure 2g | 0.5 | 0.2 | 0.05 | 0.25 | 12 well |
| Supplementary Figure 3a | n/a | 3 | n/a | n/a | 6 well |
| Supplementary Figure 3b | n/a | 3 | n/a | n/a | 6 well |
| Supplementary Figure 4a | n/a | 3 | n/a | n/a | 6 well |
| Supplementary Figure 4b | n/a | 3 | n/a | n/a | 6 well |
| Supplementary Figure 4c | n/a | 3 | n/a | n/a | 6 well |
| Supplementary Figure 4d | n/a | n/a | n/a | n/a | 12 well |
| Supplementary Figure 5a | n/a | 36 | n/a | n/a | 15 cm |

**Supplementary Note for Chemical Synthesis (A) The general procedures for CEM synthesis. (B) Chemical synthesis and characterization of CEM87. (C) Chemical synthesis and characterization of CEM88. (D) Chemical synthesis and characterization of CEM114.**

**A. Chemistry General Procedures.** FK506 was purchased from Selleckchem. All other chemicals were purchased from Sigma-Aldrich or Fisher Scientific. HPLC spectra for all compounds were acquired using an Agilent 1200 Series system with DAD detector. Analytical HPLC chromatography was performed on a 2.1×150 mm Zorbax 300SB-C18 5 μm column with water containing 0.1% formic acid as solvent A and acetonitrile containing 0.1% formic acid as solvent B at a flow rate of 0.4 mL/min. The gradient program was as follows: 1% B (0−1 min), 1−99% B (1−4 min), and 99% B (4−8 min). High resolution mass spectra (HRMS) data were acquired in positive ion mode using an Agilent G1969A API-TOF with an electrospray ionization (ESI) source. Flash column chromatography was performed on a Teledyne ISCO CombiFlash Rf system equipped with a variable wavelength UV detector and a fraction collector using RediSep Rf normal phase silica columns. Microwave reactions were performed using a Discover SP CEM. Nuclear Magnetic Resonance (NMR) spectra were acquired on a Bruker DRX-600 spectrometer with 600 MHz for proton ($^1$H NMR) and 150 MHz for carbon ($^{13}$C NMR); chemical shifts are reported in ppm (δ). Preparative HPLC was performed on Agilent Prep 1200 series with UV detector set to 254 nm. Samples were injected onto a Phenomenex Luna 75 x 30 mm, 5 μm, C18 column at room temperature. The flow rate was 30 mL/min. A linear gradient was used with 10% (or 50%) of MeOH (A) in H2O (with 0.1 % TFA) (B) to 100% of MeOH (A). HPLC was used to establish the purity of target compounds. All final compounds had > 95% purity using the HPLC methods described above.

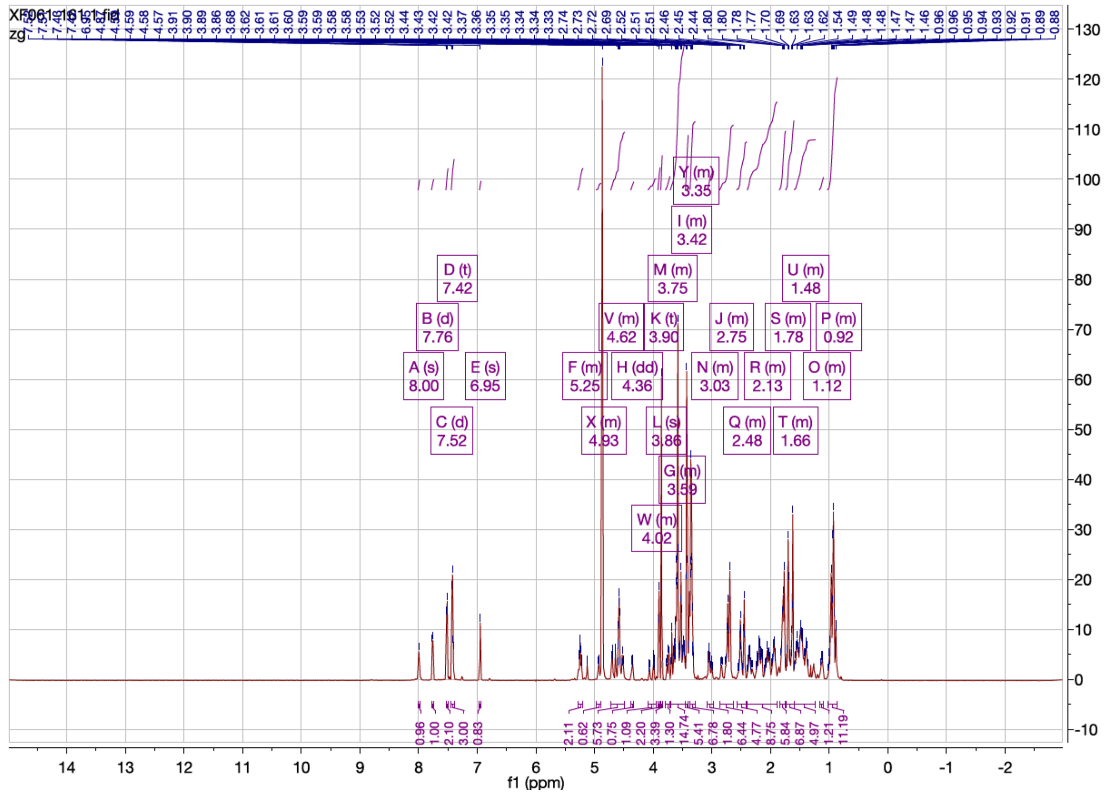


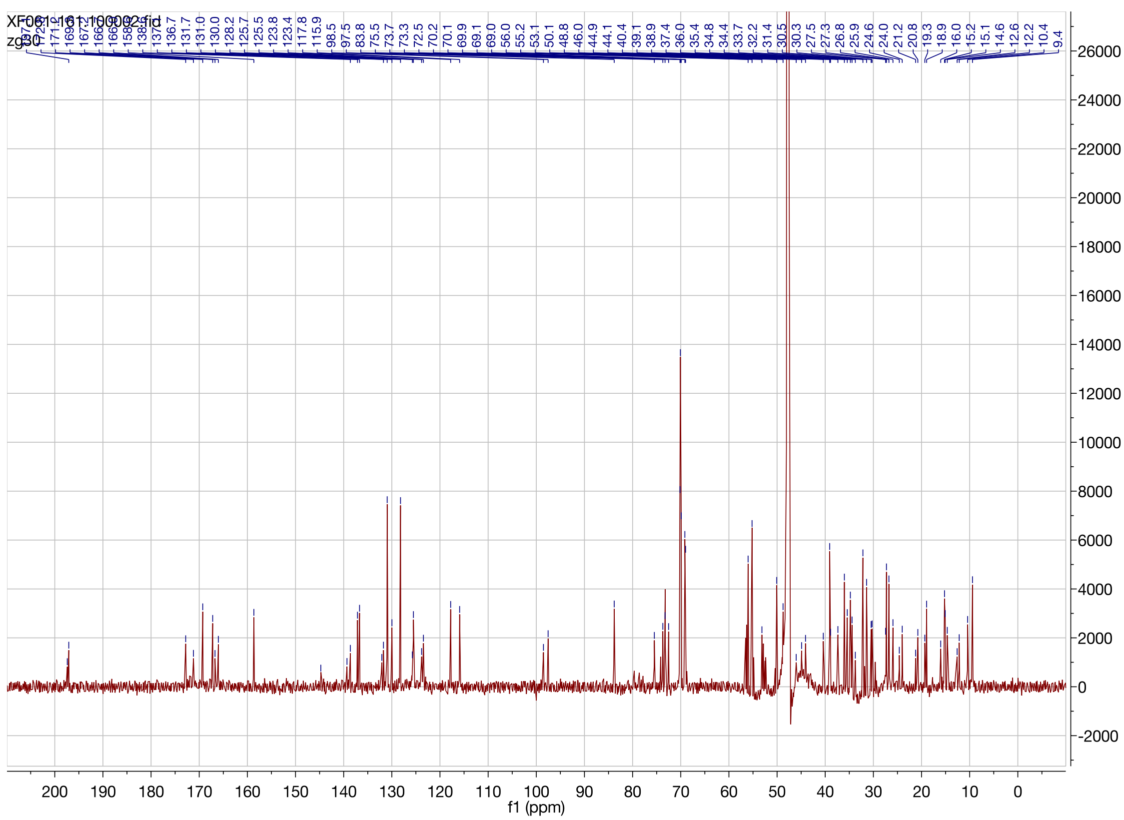

**B. (*S*)-2-(6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(prop-2-yn-1-yl)acetamide:** (*S*)-2-(6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)acetic acid (purchased from a commercial vendor) (35 mg, 0.09 mmol), propargylamine (23 μL, 0.36 mmol), EDCI-HCl (21 mg, 0.13 mmol), HOBT (25 mg, 0.18 mmol), and DIPEA 31 μL, 0.18 mmol) were all added to 4 mL dichloromethane (DCM) and stirred 24 h. The product was purified by HPLC. Yield: 35 mg, 90%. $^1$H NMR (600 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.9 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.26 - 7.23 (m, 1H), 6.90 (d, *J* = 2.8 Hz, 1H), 4.67 (t, *J* = 7.0 Hz, 1H), 4.23 - 3.98 (m, 2H), 3.83 (s, 3H), 3.52 - 3.39 (m, 2H), 2.70 (s, 3H). TOF-HRMS (m/z) $[M+H]^+$ for $C_{23}H_{21}ClN_5O_2^+$ calculated 434.1378, found 434.1396.

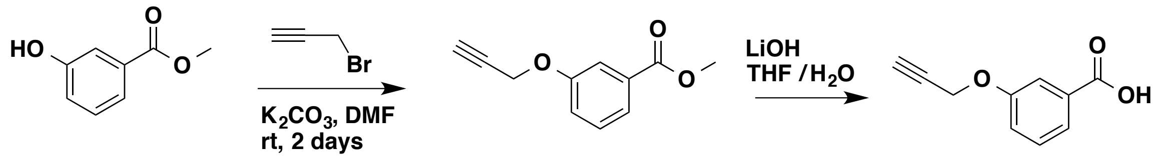


**B.** **CEM87:** (*S*)-2-(6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(prop-2-yn-1-yl)acetamide (15 mg, 0.035 mmol), compound **A** (shown above, synthesized as previously described, *ACS Synth. Biol.*, 38-45, 2018) (39 mg, 0.035 mmol), were dissolved in 1 mL *tert*-butanol. TBTA (2 mg), copper sulfate pentahydrate (0.05 equiv of a 0.1M solution in water), and sodium ascorbate (0.2 equiv of a 0.1M solution in water) were added to the solution. The reaction was stirred for 24 h then purified by HPLC. Yield: 8 mg, 15%. $^{1}$H NMR (800 MHz, MeOD) δ 8.00 (s, 1H), 7.76 (d, *J* = 8.9 Hz, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.42 (t, *J* = 8.0 Hz, 3H), 6.95 (s, 1H), 5.28 – 5.20 (m, 2H), 4.97 – 4.89 (m, 1H), 4.72 – 4.49 (m, 6H), 4.36 (dd, *J* = 13.9, 4.0 Hz, 1H), 4.09 – 3.95 (m, 1H), 3.90 (t, *J* = 5.2 Hz, 2H), 3.86 (s, 3H), 3.79 – 3.72 (m, 1H), 3.71 – 3.46 (m, 15H), 3.45 – 3.40 (m, 5H), 3.37 – 3.29 (m, 9H), 3.09 – 2.97 (m, 2H), 2.86 – 2.64 (m, 6H), 2.57 – 2.41 (m, 5H), 2.39 – 1.89 (m, 9H), 1.85 – 1.74 (m, 6H), 1.74 – 1.60 (m, 7H), 1.59 – 1.24 (m, 5H), 1.16 – 1.10 (m, 1H), 1.02 – 0.86 (m, 11H). $^{13}$C NMR (201 MHz, MeOD) δ 197.4, 197.1, 172.8, 171.2, 169.3, 167.2, 166.7, 166.0, 158.6, 144.7, 139.3, 138.6, 137.1, 136.7, 132.1, 131.7, 131.0, 130.0, 128.2, 125.7, 125.5, 123.8, 123.4, 117.8, 115.9, 98.5, 97.5, 83.8, 75.5, 73.7, 73.3, 72.5, 70.2, 70.1, 69.9, 69.1, 69.0, 56.0, 55.2, 53.1, 50.1, 48.8, 46.0, 44.9, 44.1, 40.4, 39.1, 38.9, 37.4, 36.0, 35.4, 34.8, 34.4, 33.7, 32.2, 31.4, 30.5, 30.3, 27.5, 27.3, 26.8, 25.9, 24.6, 24.0, 21.2, 20.8, 19.3, 18.9, 16.0, 15.2, 15.1, 14.6, 12.6, 12.2, 10.4, 9.4. TOF-HRMS (m/z) $[M+H]^+$ for $C_{78}H_{112}ClN_{10}O_{18}S^+$ calculated 1543.7560, found 1543.7565. HPLC $t_R$ = 4.78 min.

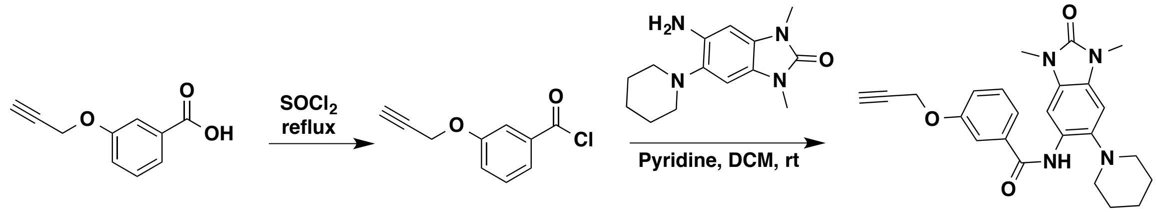
# 1H NMR and 13C NMR Spectra of CEM87:



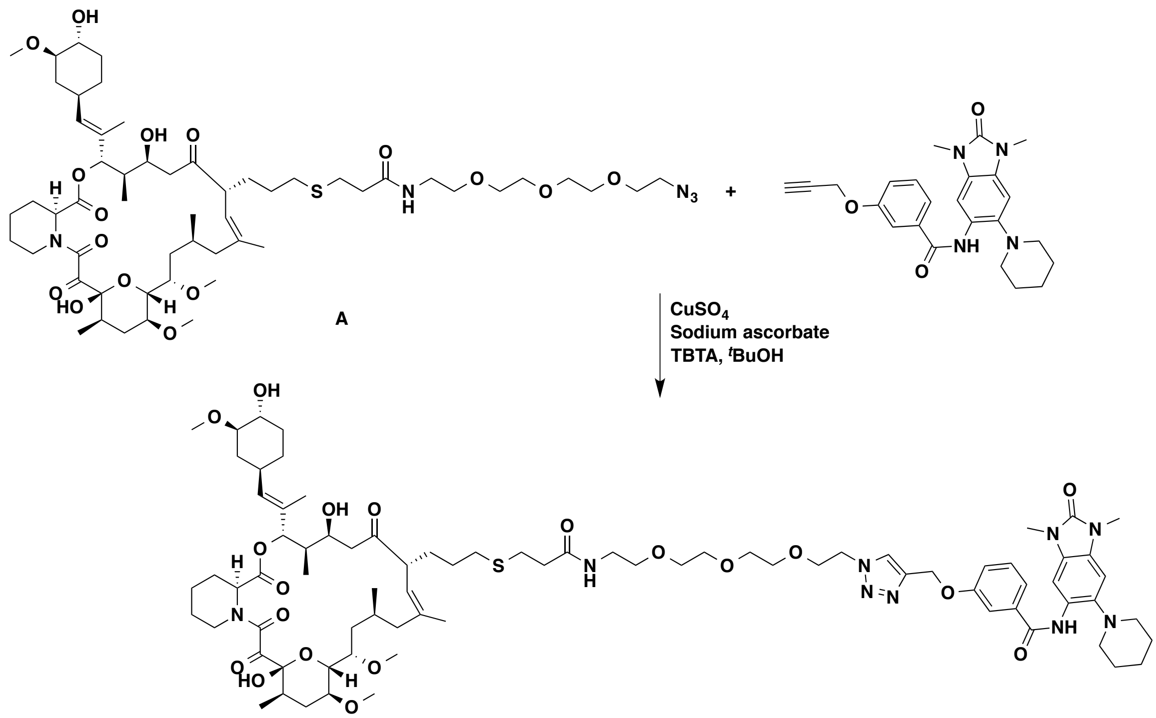




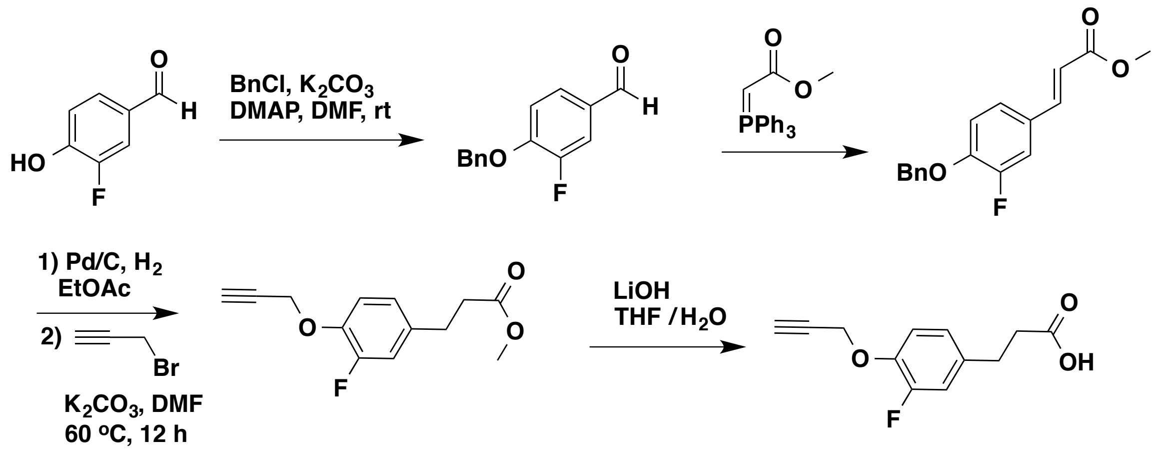

**C. 3-(prop-2-yn-1-yloxy)benzoic acid:** To the solution of methyl 3-hydroxybenzoate (760 mg, 5 mmol) in DMF (10 mL) was added $K_2CO_3$ (1.38 g, 10 mmol). Then, 3-bromoprop-1-yne (1.49 mL, 10 mmol) was added, and the reaction was stirred at room temperature for 2 days. Water and ethyl acetate (EtOAc) were added, and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water, brine, and dried over $Na_2SO_4$. The solvent was removed and dried under vacuum. The crude product (950 mg, 5 mmol), which was used for next step without further purification, was dissolved in THF / $H_2O$ (25 mL / 25 mL). Then, LiOH (1.0 g, 43 mmol) was added, and the reaction was stirred at room temperature overnight. The reaction mixture was then treated with 4 N HCl until pH = 1, and extracted with EtOAc (3 x 30 mL), dried over $Na_2SO_4$. The solvent was removed under reduced pressure to obtained the title compound (801 mg, yield 91% over two steps). $^1$H NMR (600 MHz, Chloroform-*d*) δ 7.79 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.73 (dd, *J* = 2.9, 1.5 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.30 – 7.20 (m, 1H), 4.78 (d, *J* = 2.4 Hz, 2H), 2.58 (t, *J* = 2.3 Hz, 1H).



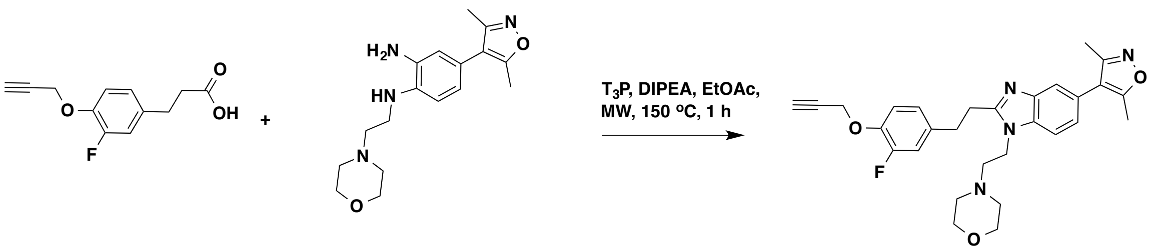

**C. *N*-(1,3-dimethyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-(prop-2-yn-1-yloxy)benzamide:** 3-(prop-2-yn-1-yloxy)benzoic acid (200 mg, 1.13 mmol) was dissolved in excess $SOCl_2$, and the reaction was heated to reflux for 2 h. Then, the mixture was cooled and concentrated to generate 3-(prop-2-yn-1-yloxy)benzoyl chloride for the next step without purification. To a solution of 5-amino-1,3-dimethyl-6-(piperidin-1-yl)-1H-benzo[*d*]imidazole-2(3H)-one (shown above, synthesized as previously described, *ACS Med. Chem.*, 1190-1195, 2014) (323 mg, 1.24 mmol) in $CH_2Cl_2$ (10 mL) was added pyridine (0.114 mL, 1.41 mmol) at room temperature. Then, 3-(prop-2-yn-1-yloxy)benzoyl chloride (220 mg, 1.13 mmol) was added and the reaction mixture was stirred for 1 h at this temperature. The reaction was diluted with DCM and washed by saturated $NaHCO_3$ solution, and the aqueous phase was extracted with DCM. The combined organics were washed with water, dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (50%-100% of EtOAc in Hexanes), and to obtain the product as white solid (294 mg, yield 62%). $^1$H NMR (600 MHz, Methanol-*d*$_4$) δ 8.19 (s, 1H), 7.57 – 7.45 (m, 3H), 7.29 – 7.22 (m, 1H), 7.16 (s, 1H), 4.84 (d, *J* = 2.4 Hz, 2H), 3.41 (d, *J* = 4.8 Hz, 6H), 3.01 (t, *J* = 2.4 Hz, 1H), 2.88 (t, *J* = 5.2 Hz, 4H), 1.79 (p, *J* = 5.7 Hz, 4H), 1.65 (brs, 2H). HRMS (m/z) $[M + H]^+$ for $C_{24}H_{27}N_4O_3^+$ calculated 419.2078, found 419.2155.



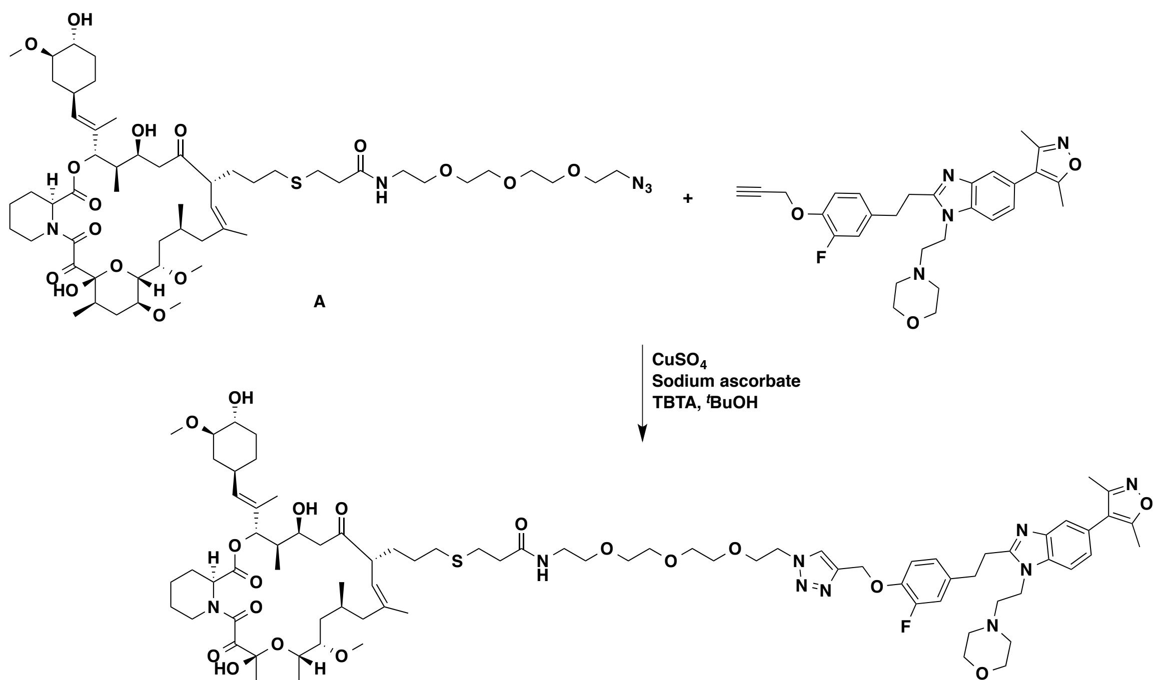
**C. CEM88:** Compound A[1] (39 mg, 0.035 mmol) and *N*-(1,3-dimethyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-(prop-2-yn-1-yloxy)benzamide (15 mg, 0.035 mmol) were dissolved in *tert*-Butanol (1 mL). TBTA (2 mg, 10 mol %), copper sulfate pentahydrate (0.035 mL, 10 mol %, 0.1 M), and sodium ascorbate (0.17 mL, 0.2 equiv.) were added, and the reaction was stirred at room temperature for 24 h. The reaction mixture was purified by preparative HPLC (10%-100% methanol / 0.1% TFA in $H_2O$) to afford CEM88 as white solid in TFA salt form (38 mg, yield 71%). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 8.19 (s, 1H), 7.83–7.57 (m, 2H), 7.55–7.44 (m, 2H), 7.36–7.30 (m, 2H), 5.30 (s, 2H), 5.24–5.08 (m, 2H), 4.63–4.58 (m, 3H), 4.33 (d, *J* = 13.6 Hz, 1H), 4.06–3.87 (m, 3H), 3.74–3.67 (m, 1H), 3.63–3.53 (m, 11H), 3.55–3.36 (m, 19H), 3.07–2.92 (m, 2H), 2.79 (dd, *J* = 14.4, 5.8 Hz, 1H), 2.68 (t, *J* = 7.2 Hz, 2H), 2.46 (s, 2H), 2.40 (t, *J* = 7.3 Hz, 2H), 2.37–2.23 (m, 2H), 2.21–2.07 (m, 3H), 2.06–1.68 (m, 18H), 1.67–1.56 (m, 8H), 1.56–1.26 (m, 10H), 1.25–1.16 (m, 1H), 1.09 (q, *J* = 12.9, 12.4 Hz, 1H), 0.99–0.79 (m, 11H). HRMS (m/z) $[M+H]^+$ for $C_{79}H_{118}N_9O_{19}S^+$ calculated 1528.8259, found 1528.8327. HPLC $t_R$ = 4.76 min.



**D. 3-(3-fluoro-4-(prop-2-yn-1-yloxy)phenyl)propanoic acid**: To a solution of 3-fluoro-4-hydroxybenzaldehyde (1.40 g, 10 mmol) in dimethyl formamide (DMF) (20 mL) was added benzyl chloride (1.39 mL, 12 mmol), $K_2CO_3$ (2.04 g, 15 mmol), and DMAP (10 mg). The reaction was stirred overnight at room temperature. Water and EtOAc were added, and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water, brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (0%-10% of EtOAc in Hexane). The product 4-(benzyloxy)-3-fluorobenzaldehyde was obtained as white solid (1.33 g, yield 58%). 4-(benzyloxy)-3-fluorobenzaldehyde (1.33 g, 5.8 mmol) and methyl (triphenylphosphoranylidene) acetate (2.12 g, 6.4 mmol) were dissolved in DCM (20 mL). The resulting mixture was stirred at room temperature for 3 days. The solvent was removed and purified by flash chromatography on silica gel (0%-25% of EtOAc in Hexane). Methyl 3-(4-(benzyloxy)-3-fluorophenyl)acrylate was obtained as white solid (1.55 g, yield 93%) and dissolved in EtOAc (50 mL). Pd/C (200 mg) was added and the reaction was stirred under $H_2$ for 18 h. The Pd/C was filtered through Celite, and the filtrate was concentrated. The crude product was dissolved in DMF (20 mL). Then, 3-bromoprop-1-yne (0.72 mL, 6.5 mmol) and $K_2CO_3$ (1.1 g, 8.1 mmol) were added. The reaction was stirred at 60 °C overnight. Water and EtOAc were added, and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water, brine, and dried over $Na_2SO_4$. The solvent was removed and purified by flash chromatography on silica gel (0%-50% of EtOAc in Hexane). Methyl 3-(3-fluoro-4-(prop-2-yn-1-yloxy)phenyl)propanoate was obtained as white solid (892 mg, yield 70%). Methyl 3-(3-fluoro-4-(prop-2-yn-1-yloxy)phenyl)propanoate (850 mg, 3.6 mmol) was dissolved in THF/$H_2O$ (8 mL/8 mL). LiOH (432 mg, 18 mmol) was added, and the reaction was stirred at room temperature overnight. The reaction was treated with 2 N HCl until pH = 1, extracted with EtOAc (3 x 30 mL), dried over $Na_2SO_4$. The solvent was removed to get the title product (754 mg, yield 94%). $^1H$ NMR (600 MHz, Methanol-$d_4$) δ 7.10 (t, $J$ = 8.5 Hz, 1H), 7.00 (dd, $J$ = 25.3, 10.2 Hz, 2H), 4.77 (q, $J$ = 3.5 Hz, 2H), 3.02 – 2.92 (m, 1H), 2.87 (d, $J$ = 7.4 Hz, 2H), 2.64 – 2.54 (m, 2H).



**D. 4-(2-(5-(3,5-dimethylisoxazol-4-yl)-2-(3-fluoro-4-(prop-2-yn-1-yloxy)phenethyl)-1*H*-benzo[*d*]imidazol-1-yl)ethyl)morpholine:** 3-(3-fluoro-4-(prop-2-yn-1-yloxy)phenyl)propanoic acid (53 mg, 0.24 mmol) and 4-(3,5-dimethylisoxazol-4-yl)-$N^1$-(2-morpholinoethyl)benzene-1,2-diamine (shown above, synthesized as previously described, *J. Am. Chem. Soc.,* 9308-9319, 2014) (63 mg, 0.2 mmol) were dissolved in EtOAc (0.7 mL). $T_3P$ (0.578 mL, 1 mmol) and DIPEA (0.041 mL, 0.24 mmol) were added, then, the reaction was heated to 150 $^oC$ for 10 minutes in microwave reactor (MW). Water was added to quench the reaction, and the aqueous phase was extracted with EtOAc (3 x 5 mL), dried over $Na_2SO_4$. Solvent was removed and the resulting mixture was purified by preparative HPLC (10%-100% methanol / 0.1% TFA in $H_2O$) to afford title compound as white solid in TFA salt form (41 mg, yield 41%). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 8.04 (d, *J* = 8.6 Hz, 1H), 7.74 (d, *J* = 1.4 Hz, 1H), 7.60 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.19 – 7.11 (m, 2H), 7.03 (d, *J* = 8.3 Hz, 1H), 4.78 (d, *J* = 2.4 Hz, 2H), 3.94 (s, 4H), 3.58 (t, *J* = 7.8 Hz, 2H), 3.54 – 3.48 (m, 2H), 3.35 (d, *J* = 9.1 Hz, 4H), 3.31 (p, *J* = 1.6 Hz, 4H), 2.98 (t, *J* = 2.5 Hz, 1H), 2.44 (s, 3H), 2.27 (s, 3H). HRMS (m/z) $[M + H]^+$ for $C_{29}H_{32}FN_4O_3^+$ calculated 503.2453, found 503.2442.



**D. CEM114**: CEM114 was synthesized following the standard procedures for preparing CEM88 from Compound A[1] (36 mg, 0.032 mmol) and 4-(2-(5-(3,5-dimethylisoxazol-4-yl)-2-(3-fluoro-4-(prop-2-yn-1-yloxy)phenethyl)-1*H*-

benzo[*d*]imidazol-1-yl)ethyl)morpholine (20 mg, 0.032 mmol), TBTA (1 mg, 10 mol %), copper sulfate pentahydrate (0.032 mL, 10 mol %, 0.1 M), and sodium ascorbate (0.16 mL, 0.2 equiv) in *tert*-Butanol (1 mL). The reaction mixture was purified by preparative HPLC (10%-100% methanol / 0.1% TFA in $H_2O$) to afford CEM114 as white solid in TFA salt form (23 mg, yield 45%). $^1H$ NMR (600 MHz, Methanol-$d_4$) δ 8.16 (d, *J* = 2.8 Hz, 1H), 7.98 (dd, *J* = 8.6, 3.2 Hz, 1H), 7.72 (s, 1H), 7.58 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.19 (td, *J* = 8.5, 5.3 Hz, 1H), 7.15 – 7.10 (m, 1H), 7.01 (t, *J* = 8.6 Hz, 1H), 5.24 – 5.12 (m, 4H), 4.66 – 4.57 (m, 3H), 4.28 (d, *J* = 13.4 Hz, 1H), 4.06 – 3.94 (m, 1H), 3.89 (dd, *J* = 12.9, 7.8 Hz, 7H), 3.75 – 3.66 (m, 1H), 3.63 – 3.51 (m, 13H), 3.48 (t, *J* = 5.5 Hz, 3H), 3.43 – 3.36 (m, 8H), 3.26 – 3.11 (m, 7H), 3.01 (ddd, *J* = 15.0, 9.6, 4.1 Hz, 1H), 2.92 (t, *J* = 12.4 Hz, 1H), 2.80 (dd, *J* = 14.3, 5.3 Hz, 1H), 2.70 (t, *J* = 7.2 Hz, 2H), 2.51 – 2.40 (m, 8H), 2.28 (s, 5H), 2.23 – 1.97 (m, 6H), 1.95 – 1.68 (m, 9H), 1.68 – 1.30 (m, 17H), 1.24 – 1.14 (m, 1H), 1.13 – 1.04 (m, 1H), 1.00 – 0.77 (m, 11H). HRMS (m/z) $[M+H]^+$ for $C_{84}H_{123}N_9O_{19}S^+$ calculated 1612.8634, found 1612.8475. HPLC $t_R$ = 4.27 min.