Supplementary sequences

5`-
CAGCCACTCGAGGATCCGGATTAGTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGACTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGA
TAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCGGCCCTACGTGCTGTCTCACACAGCCTGTCTGACGGCCCT
ACGTGCTGTCTCACACAGCCTGTCTGACGGCCCTACGTGCTGTCTCACACAGCCTGTCTGACGGCCCTACGTGCTGTCTCACACAGCCTGTCTGACGGCC
AGCCTGTCTGACGGCCCTACGTGCTGTCTCACACAGCCTGTCTGACGGCCCTACGTGCTGTCTCACACAGCCTGTCTGACGGCCCTACGTGCTGTCTCACAC
CTACGTGCTGTCTCACACAGCCTGTCTGACTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCT
CGCTTCTCGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAAC
CCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACACATGCAGCATGTATCAAAATTAATTT
GGTTTTTTTTCTTAAGTATTTACATTAAATGGCCATAGTACTTAAAGTTACATTGGCTTCCTTGAAATAAACATGGAGTATTCAGAATGTGTCATAAATATTTCTAATTTTAAGATAGTA
TCTCCATTGGCTTTCTACTTTTTCTTTTATTTTTTTTTGTCCTCTGTCTTCCATTTGTTGTTGTTGTTGTTTGTTTGTTTGTTTGTTGGTTGGTTGGTTAATTTTTTTTTAAAGATCCTACACT
ATAGTTCAAGCTAGACTATTAGCTACTCTGTAACCCAGGGTGACCTTGAAGTCATGGGTAGCCTGCTGTTTTAGCCTTCCCACATCTAAGATTACAGGTATGAGCTATCATTTTTGGT
ATATTGATTGATTGATTGATTGATGTGTGTGTGTGTGATTGTGTTTGTGTGTGTGACTGTGAAAATGTGTGTATGGGTGTGTGTGAATGTGTGTATGTATGTGTGTGTGTGAGTGTG
TGTGTGTGTGTGCATGTGTGTGTGTGACTGTGTCTATGTGTATGACTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTGTGAAAAAATATTCTATGG
TAGTGAGAGCCAACGCTCCGGCTCAGGTGTCAGGTTGGTTTTTGAGACAGAGTCTTTCACTTAGCTTGGAATTCACTGGC-3'

Annotations:

<u>3'-LTR</u>

Enzyme Recognition Site

NheI restriction site

XbaI restriction site

HRE Repeats (9x)

HBS (HIF-binding site)

6b.p. overhang (for efficient restriction endonuclease cleavage)

Figure S1. Synthetic nine tandem HRE fragment sequence. Related to Fig. 1.

Panel A b.p. sequence

5`-AGACTGCCATGG ...
GCCCCAGCCGCTGGAGACACAATCATATCTTTAGATTTTGGCAGCAACGACACAGAAACTGATGACCAGCAACTTGAGGAAGTACCATTATATAATGATGTAATGCTCCCCTCACCC
AACGAAAAATTACAGAATATAAATTTGGCAATGTCTCCATTACCCACCGCTGAAACGCCAAAGCCACTTCGAAGTAGTGCTGACCCTGCACTCAATCAAGAAGTTGCATTAAAATTA
GAACCAAATCCAGAGTCACTGGAACTTTCTTTTACCATGCCCCAGATTCAGGATCAGACACCTAGTCCTTCCGATGGAAGCACTAGACAAAGTTCACCTGAGCCTAATAGTCCCAGT
GAATATTGTTTTTATGTGGATAGTGATATGGTCAATGAATTCAAGTTGGAATTGGTAGAAAAACTTTTTGCTGAAGACACAGAAGCAAAGAACCCATTTTCTACTCAGGACACAGAT
TTAGACTTGGAGATGTTAGCTCCCTATATCCCAATGGATGATGACTTCCAGTTACGTTCCTTCGATCAGTTGTCACCATTAGAAAGCAGTTCCGCAAGCCCTGAAAGCGCAAGTCCTC
AAAGCACAGTTACAGTATTCCAGTAACAGCCACTCGAGGATCCG-3′

Annotations:

Enzyme Recognition Site

Start codon

... = CAR or protein of interest sequence (left to right, from N- to C-terminus)

ODD

EcoRI site (unused) and suggested b.p. to mutate to remove if it will pose an issue for downstream cloning

Stop codon

6b.p. overhang (for efficient restriction endonuclease cleavage)

Panel B amino acid sequence

APAAGDTIISLDFGSNDTETDDQQLEEVPLYNDVMLPSPNEKLQNINLAMSPLPTAETPK
PLRSSADPALNQEVALKLEPNPESLELSFTMPQIQDQTPSPSDGSTRQSSPEPNSPSEYC
FYVDSDMVNEFKLELVEKLFAEDTEAKNPFSTQDTDLDLEMLAPYIPMDDDFQLRSFDQL
SPLESSSASPESASPQSTVTVFQ

Figure S2. ODD sequences. Related to Fig. 3. **A**) Synthetic ODD fragment DNA sequence. The boxed codon within the purple ODD region (GAA) represented part of an EcoRI site that may cause issues should the user need to further modify the HRE cassette after incorporating the CAR-ODD into the SFG vector (as the HRE promoter insertion uses and EcoRI cleavage site). Should this potentially be needed, we recommend mutating the highlighted site to [GAG] to avoid the EcoRI site but maintain the a.a. sequence of the ODD protein when translated. **B**) Amino acid sequence of human HIF1α ODD (a.a. 401-603).