YMTHE, Volume 25

Supplemental Information

A Fusion Receptor as a Safety Switch, Detection,

and Purification Biomarker for Adoptive

Transferred T Cells

Xiuqi Wu, Bizhi Shi, Jiqin Zhang, Zhimin Shi, Shengmeng Di, Minliang Fan, Huiping Gao, Hai Wang, Jianren Gu, Hua Jiang, and Zonghai Li

Supplemental Table

Table S1.

Amino acid sequence of FR806:

maqrmttqllllvwvavvgeaqtvracgadsyemeedgvrkckkriawartellnvcmnakhhkekpgpedklheqcrpwrknaccstnt sqeahkdvsylyrfnwnhcgemapackrhfiqdtclyecspnlgpwiqqvdqswrkervlnvplckedceqwwedcrtsytcksnwhkg wnwtsgfnkcavgaacqpfhfyfptptvlcneiwthsykvsnysrgsgrciqmwfdpaqgnpneevarfyaaamsgagpwaawpfllslal mllwlls

Ribosomal skipping sequence (F2A):

vkqtlnfdllklagdvesnpgp

Amino acid sequence of anti-CD19 CAR:

malpvtalllplalllhaarpdiqltqspaslavslgqratisckasqsvdydgdsylnwyqqipgqppklliydasnlvsgipprfsgsgsgtdftln ihpvekvdaatyhcqqstedpwtfgggtkleikgggssggggsggggsqvqlqqsgaelvrpgssvkisckasgyafssywmnwvkqrpg qglewigqiwpgdgdtnyngkfkgkatltadessstaymqlsslasedsavyfcarretttvgryyyamdywgqgttvtvsstttpaprpptpap tiasqplslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcelr vkfsrsadapayqqgqnqlynelnlgrreeydvldkrrgrdpemggkpqrrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyq glstatkdtydalhmqalppr

Supplemental Figures

Figure S1:



Figure S1: CH12 and CH12-MMAF stained FR806⁺ **T-cells with equal intensity**. T cells transduced with FR806 were stained with CH12 and CH12-MMAF at the concentration of 10µg /ml followed by goat anti-human IgG-PE. The grey line shows staining with an isotype IgG antibody followed by goat anti-human IgG-PE.

Figure S2:



Figure S2: Confocal microscopy confirmed the internalization of CH12-MMAF into FR806⁺ T-cells. FR806⁺ T-cells were treated with CH12-MMAF for 4h at $37 \degree$ C. After fixation and permeabilization, the cells were stained with a PE-conjugated secondary antibody and DAPI. Scale bar

represents 20 µM.

Figure S3:



Figure S3: Titration of biotinylated CH12. FR806⁺ T cells were incubated with biotinylated CH12 at the indicated concentrations and subsequently labeled with the secondary antibody streptavidin-PE.

Figure S4:



Figure S4: Cytotoxity of CH12-MMAF to keratinocytes. Keratinocytes were exposed to various concentrations of CH12-MMAF for 72h followed by a cell viability assay. Percentage survival (mean ±SD) of triplicate wells is depicted.