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Supplemental Material to:

Yael Luria, Dina Raichlin and Itai Benhar

Fluorescent IgG fusion proteins made in *E. coli*

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Supplementary Methods

Construction of plasmids for the production of mCherry-fusion antibodies. Plasmids for the expression of the red fluorescent protein mCherry fused to the C-terminus of human gamma1 Fc and human kappa light chain constant region were constructed similarly to the SFGFP vectors described in the materials and methods section of the article. The coding sequence of mCherry was obtained by PCR amplification using the plasmid pmCherry (Clontech, USA) as template with primers mCherry-HindIII-FOR (CGGGTAAAGCTTCCGGAGGTGTGAGCAAGGGCGAGGAG) and mCherry-EcoRI-REV (AGCAGCGAATTCTTACTTGTACAGCTCGTCCATGC). The resulting PCR product (720 bp) was digested with *Hind*III and *Eco*RI and was ligated with vector fragments that were recovered after digesting plasmids pHAK-IgH-FRP5-PE38 (4380 bp vector fragment) and pHAK-IgL-FRP5-PE38 (3700bp vector fragment) [1] with the same enzymes. The resulting plasmids were named pHAK-IgH-FRP5-mCherry and pHAK-IgL-FRP5-mCherry.

Production of mCherry-fusion antibodies. All the mCherry-fused IgGs were prepared according to the Inclonals protocol as previously described. [1]

FACS analysis of cell staining with mCherry-fused FRP5 IgG. Approximately 5×10^5 cells were taken for each FACS experiment. The cells were trypsin-detached, washed with FACS buffer (2% FCS in PBS) and incubated with protein A purified IgGs (34.25 nM/3% BSA in PBS) at 4°C for 1 h. The cells were then washed twice with FACS buffer to remove unbound antibodies. For binding capacity evaluation, the cells were next incubated with FITC-conjugated $F(ab')_2$ –specific Goat Anti-Human IgG (H+L) (Jackson ImmunoResearch Laboratories) (1:500/3% BSA in PBS) at 4°C for 30 minutes. After another washing step, 10,000 cells were analyzed by flow cytometer (FACSAria Flow Cytometer) using Octagon
488-nm blue laser (mCherry and FITC excitation). Data was analyzed using FlowJo 7.6.5 Multi-platform.

Measuring refolding kinetics of SFGFP. Antibody light chain-SFGFP fusion protein was expressed as cytoplasmic inclusion bodies and inclusion bodies were prepared as described for antibody production. DTE was added to the solution for different time periods so that the protein was reduced for 1:30, 1:45 or 2 h (2 h is the reduction time used for antibody production). Refolding was next initiated by ×100 dilution of the protein solution into a refolding buffer (which inclused oxidized glutathione). Fluorescence intensity was measured at room temperature using a Biotek Synergy plate reader with an excitation wavelength of 485/20nm and emission measured at 528/20nm. Measurements were made at time 0 (before the dilution into the refolding buffer) and every 1 minute for 40 minutes.

Supplementary figure legends

Supplementary Figure 1. Evaluation of fluorescence intensity of FRP5-tetra-tandem-SFGFP on antigen positive *vs.* negative cells. Cellular ErbB2 binding was evaluated on human ErbB2-negative Pig cell line PAE (porcine aortic endothelial cells [2]) and on ErbB2 positive human breast adenocarcinoma cell line SKBR3 without a secondary antibody (measuring the green fluorescence of the antibodies. Filled (purple) areas, negative control (auto fluorescence of cells).

Supplementary Figure 2. Evaluation of fluorescence intensity of mCherry fused FRP5 (FRP5-tetra-mCherry) by FACS. Cellular ErbB2 binding was evaluated on human ErbB2-negative Pig cell line PAE, on ErbB2-positive (low) human epithelial carcinoma cell line A431 and on ErbB2 positive (high) human breast adenocarcinoma cell line SKBR3 with (measuring green fluorescence, panels A, B, C) and without a secondary antibody (measuring the red fluorescence of the antibodies, panels D, E, F). Black lines are the negative control (auto fluorescence of cells).

Supplementary Figure 3. Analysis of refolding kinetics of SFGFP fused to FRP5 light chain.

Supplementary Figure 4. Physical and restriction map (only unique restriction sites are shown) of plasmid pHAK-IgH-SFGFP.

Supplementary Figure 5. Physical and restriction map (only unique restriction sites are shown) of plasmid pHAK-IgL-SFGFP.

Supplementary references

1. Hakim R, Benhar I. "Inclonals": IgGs and IgG-enzyme fusion proteins produced in an E. coli expression-refolding system. MAbs 2009; 1:281-7.

2. Kigel B, Rabinowicz N, Varshavsky A, Kessler O, Neufeld G. Plexin-A4 promotes tumor progression and tumor angiogenesis by enhancement of VEGF and bFGF signaling. Blood. 2011; 118(15):4285-96.

Supplementary Table 1: **PCR primers (restriction sites are underlined)**

Primer name	Sequences
FRP5VH-NdeI-FOR	5'ATATATCATATGCAGGTACAACTGCAGCAGTCT
FRP5VH-NheI-REV	5'ATATATGCTAGCAGAGGAAACGGTGACCGTGGTCC
FRP5VL-NdeI-FOR	5'ATATATCATATGGACATCCAGCTGACCCAGTCTCAC
FRP5VL-BsiWI-REV	5'AGCCACCGTACGTTTGATCTCCAATTTTGTCCCCGAGC
RGD/TAT-BsrGI-FOR	5'GACGTGAGCCACGAAGACCCTGAGGTC
CH3-HindIII-EcoRI-REV	5'AAATTTGAATTCACCTCCGGAAGCTTTACCCGGGGACAGGGAG
BsiWI-Back-IgL	5'AAACGGCGTACGGTGGCTGCACCATCTGTCTTC
Cκ -HindIII-EcoRI-REV	5'AAATTTGAATTCACCTTCGGAAGCTTTTCCACCGCCACACTCT CCCCTGTTGAAG
tandem-HindIII-FOR	5'ATTACGAGTAAAGCTTCCGCTGGCTCCGCTGCTGGTTCTGGCG CAGCGGCAGTGAGCAAGGGCGAGGAGCTG
tandem-NotI-REV	5'AATTCTCACCGCGGCCGCGCTGCCGCCGCCGCCAGAACCACCA CCACCCTTGTACAGCTCGTCCATGCC