Supporting Information

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SI Materials and Methods

Cell Culture. TF-1 cells were maintained in RPMI-1640 containing 10% (vol/vol) FBS, penicillin, streptomycin, and 2 ng/mL granulocyte–macrophage colony-stimulating factor (GM-CSF). HEK293T cells were grown in DMEM containing 10% (vol/vol) FBS, penicillin, and streptomycin. HEK293F cells were maintained in FreeStyle 293 expression media with 4 mM GlutaMAX. $CD34^+$ stem cells were isolated from human bone marrow using a direct immunomagnetic CD34 MicroBead labeling system (AllCells). $CD34^+$ cells were cultured in StemSpan serum-free media (SFEM) from STEMCELL Technologies supplemented with 50 ng/mL human stem cell factor, 25 ng/mL interleukin 3, and 25 ng/mL interleukin 6.

Construction of the Lentiviral Combinatorial Antibody Library. scFv genes were obtained from a naïve human combinatorial antibody library based on antibody genes from 60 different samples of human peripheral blood lymphocytes. The library diversity is 4.5×10^9 (1). scFv genes were subcloned into the lentiviral vector at the compatible asymmetric SfiI sites as described before (2).

Preparation of Lentivirus. Virus was produced in HEK293T cells by cotransfection of lentiviral vectors with the pCMVD8.91 and pVSVg viral packaging vectors at ratio of 1:1:1. Supernatants containing virus were collected at 48 h posttransfection. Cell debris was removed by centrifugation and filtering through a 0.22-μm polyethersulfone membrane filter unit from Millipore. The titer of the lentivirus preparation was determined using a Lenti-X p24 ELISA (Clontech). The virus preparations were aliquoted and frozen at −80 °C.

Transduction of TF-1 Cells with Lentivirus. Lentivirus was added to TF-1 cells in 1 mL medium containing 5 μg/mL polybrene and 2 ng/mL GM-CSF. The "spinoculation" was performed by centrifugation of the lentivirus and cell mixture at 30 °C for 90 min at $1{,}200 \times g$. The cells were incubated with lentivirus overnight at 37 °C. Excess virus was removed, and fresh medium without GM-CSF was added the next day.

Selection of Agonist Antibodies by a Colony-Forming Cell Assay Using Methylcellulose-Based Media. TF-1 cells were transduced with the lentiviral antibody library at a multiplicity of infection of 2. The TF-1 cells transduced with the antibody library were added to methylcellulose media such that the methylcellulose final concentration was 1.27% and the cell concentration was $\sim 3 \times 10^4$ cells/mL. A total of 1.5 mL of cell suspension was added to 35-mm–diameter dishes. The cells in soft agar were cultured for 2 wk. The colonies were harvested with the aid of a micromanipulator (Sutter Instruments) and lysed with lysis buffer containing protease K for 1 h at 50 °C. The antibody genes from each colony were amplified by PCR with primer pairs customized for our lentiviral vector. The amplified antibody genes were analyzed by electrophoresis and recovered. After digestion with SfiI, the genes were ligated into the lentiviral vector and transformed into XL1-Blue competent cells. Four colonies selected from each bacterial transformation were sequenced with Sanger sequencing (BATJ). The heavy-chain CDR3 sequences were extracted by VBASE2 (http://www.vbase2.org/).

TF-1 Proliferation Assay. TF-1 cells were washed twice with RPMI to remove any residual GM-CSF and plated on a 96-well plate at 1×10^4 cells per well in RPMI-1640 with 10% heat-inactivated FBS. Conditioned media from transfected cells with antibody genes, purified antibodies, PBS, or GM-CSF were added and the wells were brought to a volume of 100 μL and incubated for 72 h at 37 °C. A total of 20 μL 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) solution (CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega) was added to each well. After 2 h, the absorbance at 490 nm was measured.

Expression and Purification of scFv-Fc Proteins and Mutagenesis. For single antibodies, the antibody expression vector was transfected into HEK293F cells. Antibodies from the pooled supernatants were purified using HiTrap Protein G HP columns with an ÄKTAxpress purifier (GE). The buffer was exchanged to Dulbecco's PBS (pH 7.4) and stored at 4 °C. DNA of mutated antibody was generated from the DNA of 12-1/12-2 antibodies by the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies).

Mass Spectrometry and Data Analysis. The peptides were trapped with a trapping column (Zorbax 300SB-C18, 5 μm × 0.3 mm; Agilent) for preconcentration and desalting using solvent A (99.9% distilled water, 0.1% formic acid). Then, the trapped peptides were eluted from the trapping column directly onto a reversed-phase analytical column (length 14 cm, inner diameter 75 μm, packed with Zorbax SB-C18, 5 μm) using mobile-phase solvents (A: 99.9% distilled water, 0.1% formic acid; B: 99.9% acetonitrile, 0.1% formic acid) with the gradient. The eluent was introduced into the linear trap quadrupole mass spectrometer from a nano-ion source with a 2-kV electrospray voltage. The analysis method consisted of a full MS scan with a range of 400–2,000 m/z followed by data-dependent MS/MS on the three most intense ions from the full MS scan. The raw data from the linear trap quadrupole were searched using the IPI human FASTA database with the MASCOT (http://www.matrixscience.com/) search engine. A peptide mass tolerance of 2.0 Da and MS/MS tolerance of 0.8 Da were allowed for peptides with tryptic specificity. The database search conditions considered one missed cleavage, variable modification of oxidation of methionine, and carbamidomethylation of cysteine as a fixed modification.

Western Blot. To prepare total cell lysates, TF-1 cells or $CD34^+$ cells were washed with PBS and then lysed in lysis buffer (50 mM Hepes, pH 7.2, 150 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4, 10% glycerol, 1% Triton X-100). The lysates were then centrifuged at $20{,}000 \times g$ for 15 min at 4 °C, and the proteins were denatured in Laemmli sample buffer (5 min at 95 °C) and then separated by SDS/PAGE. The proteins were transferred to nitrocellulose membranes using the iBlot blotting system from Invitrogen and blocked in phosphate buffered saline with Tween 20 (PBST) containing 5% BSA for 30 min before being incubated with antibodies for 3 h. After washing the membranes several times with PBST, the blots were incubated with horseradish peroxidase-conjugated anti-human or anti-rabbit antibody for 1 h. The membranes were then washed with PBST and developed by ECL.

Electron Microscopy. For the transmission electron microscopy, pieces of flat embedded resin containing the cells were glued to a blank block face and 60-nm–thin sections were cut, mounted on copper slot grids coated with parlodion, and stained with uranyl acetate and lead citrate for examination on a Philips CM100 electron microscope (FEI) at 80 kV, and images were collected

using a MegaView III CCD camera (Olympus Soft Imaging Solutions). For the scanning electron microscopy (SEM), cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and a small volume was placed on a 13-mm Whatman PC filter. The cells were rinsed on the filter with PBS. The entire double filter was then clamped between nylon washers and the unit was placed in 1% osmium tetroxide. After a PBS and water wash, the entire unit was dehydrated in ethanol and placed in a critical point dryer. The nylon washers were then dismantled, the filters were separated, and both filters were mounted onto SEM stubs with carbon tape. The stubs with attached filters were then sputter-coated with iridium for subsequent examination and documentation on a Hitachi S-4800 scanning electron microscope (Hitachi High Technologies America).

Transwell Migration. $CD34^+$ cells were loaded onto the upper chamber of a FluoroBlock Multiwall Insert System (BD Biosciences) and treated with antibody or PBS in SFEM for 16 h. The lower chamber was filled with SFEM containing 150 ng/mL SDF1α. The migrated cells in the lower chamber were stained with Calcein AM fluorescent dye (Invitrogen) and quantified by fluorometer at 494/517 nm (Ex/Em).

Immunofluorescence Staining. Cells were fixed with 4% paraformaldehyde at room temperature (RT) for 20 min, blocked, and stained with 1 ng/mL phycoerythrin (PE)-conjugated anti-human CD11c antibody and DAPI for 30 min at RT. After washing with blocking solution three times for 15 min, images were collected using a confocal microscope.

Flow Cytometry. Cells were stained with PE-conjugated anti-human CD11c (Novus Biologicals) and FITC-conjugated anti-human F4/80 (Abcam) and washed with PBS. Stained cells were analyzed with an LSRII flow cytometer (Becton Dickinson).

Statistical Analysis. The data are expressed as the means ± SE. Statistical analysis was performed using the Student *t* test or by one-way analysis of variance and the post hoc test. *P* values of <0.05 were considered significant.

1. Gao C, et al. (2002) A method for the generation of combinatorial antibody libraries using pIX phage display. *Proc Natl Acad Sci USA* 99(20):12612–12616.

2. Zhang H, Wilson IA, Lerner RA (2012) Selection of antibodies that regulate phenotype from intracellular combinatorial antibody libraries. *Proc Natl Acad Sci USA* 109(39):15728–15733.

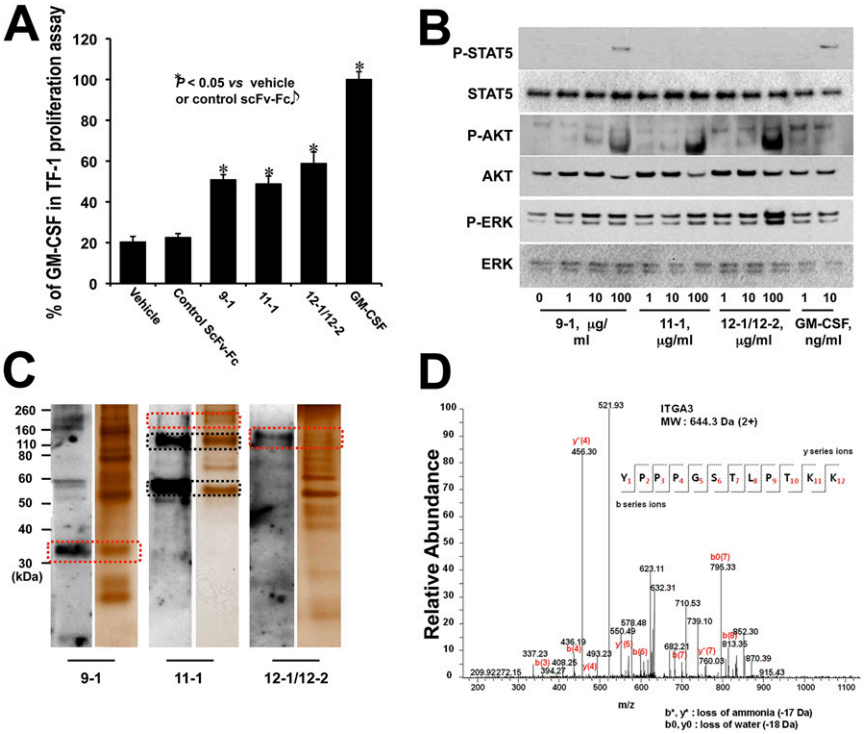

Fig. S1. Identification of targets of active antibodies. (*A*) TF-1 cells were incubated for 72 h with either purified antibodies, inactive antibodies, PBS, or GM-CSF. The number of viable cells was determined by an MTS assay. The values are the mean +/– SE of three independent experiments. **P* < 0.05 versus basal values. (*B*) TF-1 cells were incubated with several concentrations of active antibodies or GM-CSF for 10 min at 37 °C and analyzed by Western blotting using anti–p-STAT5, STAT5, p-AKT, AKT, p-ERK, and ERK antibodies. (*C*) The samples that were immunoprecipitated by active antibodies were separated by SDS/PAGE, and the gels were analyzed by Western blotting and silver staining. The red dotted boxes represent silver-stained bands that in a parallel Western blot were reactive with the antibodies. The black dotted boxes were antibody protein components. (*D*) Trypsinized gel slices from the silver-stained bands were analyzed by nano-LC-MS/MS. The MS/MS spectrum was from bands reactive with the 12-1/12-2 antibodies.

Table S1. Heavy-chain CDR3 sequences of selected cell colonies

Colony no.	Heavy-chain sequence of CDR3-DNA1	Heavy-chain sequence of CDR3-DNA2	Heavy-chain sequence of CDR3-DNA3	Heavy-chain sequence of CDR3-DNA4
2	CAREEEQQFSLDYW (2-1)	CASLDLDVW (2-2)		
3	CAREEEQQFSLDYW			
4	CARWVVGATTRIDWYFDLW (4-1)	CARDQGYSSSEGYYYYGMDVW (4-2)	CARETYYYDSSGYYYVGLFDYW (4-3)	CAREEEQQFSLDYW (4-4)
5	CAREGGSSGYDYVYYFDDW (5-1)			
6	CAREGGSSGYDYVYYFDDW (6-1)	CAREEEQQFSLDYW		
8	CARDWAKQTYCPEIYCPDAFDLW (8-1)	CVRGNSRGYYYIAGGDYS (8-2)	CARGYYRRYFDLW (8-3)	
9	CARGGDNSRGYYYIAGGDYS (9-1)			
10	CAREEEQQFSLDYW			
11	CARGGPSYGDYFRWFDPW (11-1)	CAREEEQQFSLDYW (11-2)		
12	CAREVAAAGINDAFDIW (12-1)	CARGDYYRRYFDLW (12-2)	CAREEEQQFSLDYW (12-3)	
13	CATTNPSSPLFDYW (13-1)	CAREEEQQFSLDYW (13-2)		
14	CAREEEQQFSLDYW			
15	CTRSLSPSYMYYHGMDVW (15-1)	CARGRPRDYW (15-2)		
19	CARDYYDSSGYLNTDYW (19-1)			
20	CAKDTYSSSSYFDYW (20-1)	CAREEEQQFSLDYW (20-2)		
21	CVRGNLNFLLVSW (21-1)	CAKDTYSSSSYFDYW (21-2)		
22	CAKGGGARVGATDYW (22-1)	CVRGNLNFLLVSW (22-2)		
23	CARGGLEPFDNW (23-1)	CARGTGTYLYGFCLDQW (23-2)	CARGTGTHLYGFCLDQW (23-3)	
25	CAKDSGQHRGYDFDYW (25-1)			
28	CARDRGVNWYFDLW (28-1)	CVRSGHDLWSGSTWFDPW (28-2)		

The cell colonies were numbered 1–28. Four DNAs were sequenced from each colony. The numbers in parentheses indicate the times the same sequence was repeated in the colony, as well as indicate the names of antibodies. Twenty antibody DNAs were recovered from 30 colonies.

Table S2. List of proteins from 12-1/12-2 by analysis of nano-LC-MS/MS

Accession no.	Protein name	Molecular weight	No. of identified peptides
IPI00100151	Isoform 1 of 5′-3′ exoribonuclease 2	109,494	1
IPI00292059	Nuclear pore complex protein Nup153	155,534	2
IPI00550579	cDNA FLJ12198 fis, clone MAMMA1000876	13,246	4
IPI00976712	Uncharacterized protein	105,060	2
IPI00449049	Poly[ADP ribose] polymerase 1	113,881	1
IPI00924839	Uncharacterized protein	20,007	1
IPI00335641	cDNA FLJ61243, highly similar to MKL/myocardin-like protein 2	119,335	1
IPI00029019	Isoform 2 of ubiquitin-associated protein 2-like	104,043	2
IPI00873421	Uncharacterized protein C18orf63	78,085	4
IPI00455627	Isoform 1 of Probable E3 ubiquitin-protein ligase HERC6	116,608	1
IPI00290043	Isoform 2 of integrin α3	119,952	1
IPI00976280	Conserved hypothetical protein	9,182	2
IPI00941031	cDNA FLJ12599 fis, clone NT2RM4001410	41,063	1
IPI00386617	Isoform 2 of RING finger and transmembrane domain-containing protein 2	35,656	10
IPI00012728	Isoform 1 of long-chain fatty-acid–CoA ligase 1	78,969	1
IPI00383308	HCG1648122	39,432	1
IPI00005558	Isoform 2 of Kv channel-interacting protein 2	29,284	2
IPI00478209	Isoform 1 of Ras-related protein Rab-19	24,685	2
IPI00873593	Isoform 2 of leucine-rich repeat-containing protein 16A	148,284	1
IPI00021537	Isoform 1 of opioid growth factor receptor	73,894	1

The raw data from the MS/MS analysis were searched using the IPI human FASTA database with the MASCOT search engine.