Supporting Information

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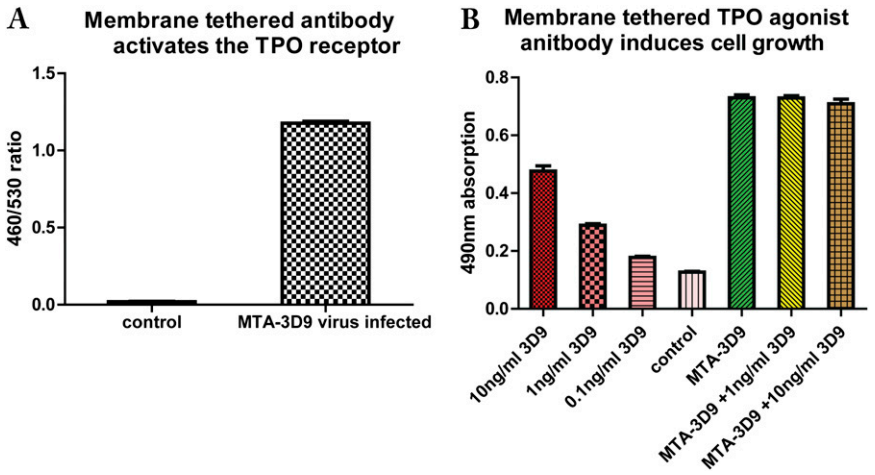


Fig. S1. Membrane-tethered thrombopoietin receptor (TPOR) agonist antibody (MTA-3D9) stimulates both reporter cell lines. (*A*) A TPOR lactamase reporter cell line is activated after infection by Lentivirus expressing the TPO agonist antibody (MTA-3D9). (*B*) A growth-based analysis. The Ba/F3–TPOR reporter cell line proliferates after infection by Lentivirus expressing antibody MTA-3D9. Cell growth was measured 2 d after virus infection and compared with treatment with the control TPO agonist antibody using a standard MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay. Each treatment was studied in triplicate. Cell growth was maximal after MTA-3D9 infection and addition of extra soluble agonist antibody had no effect.

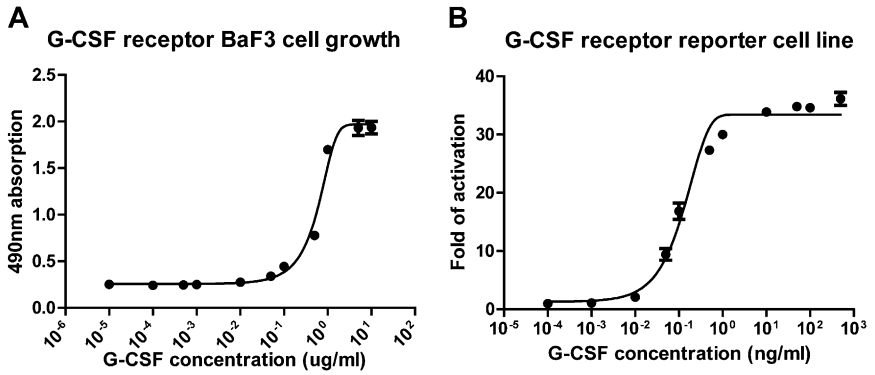


Fig. S2. Establishment of the granulocyte colony-stimulating factor (G-CSF) reporter cell lines. (*A*) The Ba/F3–G-CSF receptor (G-CSFR) reporter cell line responds by growth to G-CSF treatment in a dose dependent manner. The cell growth is determined by a standard MTS assay. (*B*) The β-lactamase based G-CSF reporter cell line (SIE/BLA/SIG) responds to different doses of G-CSF treatment. After G-CSF induction, β-lactamase activity is measured by a FRET signal generated by hydrolysis of the CCF4-AM substrate within the cells.

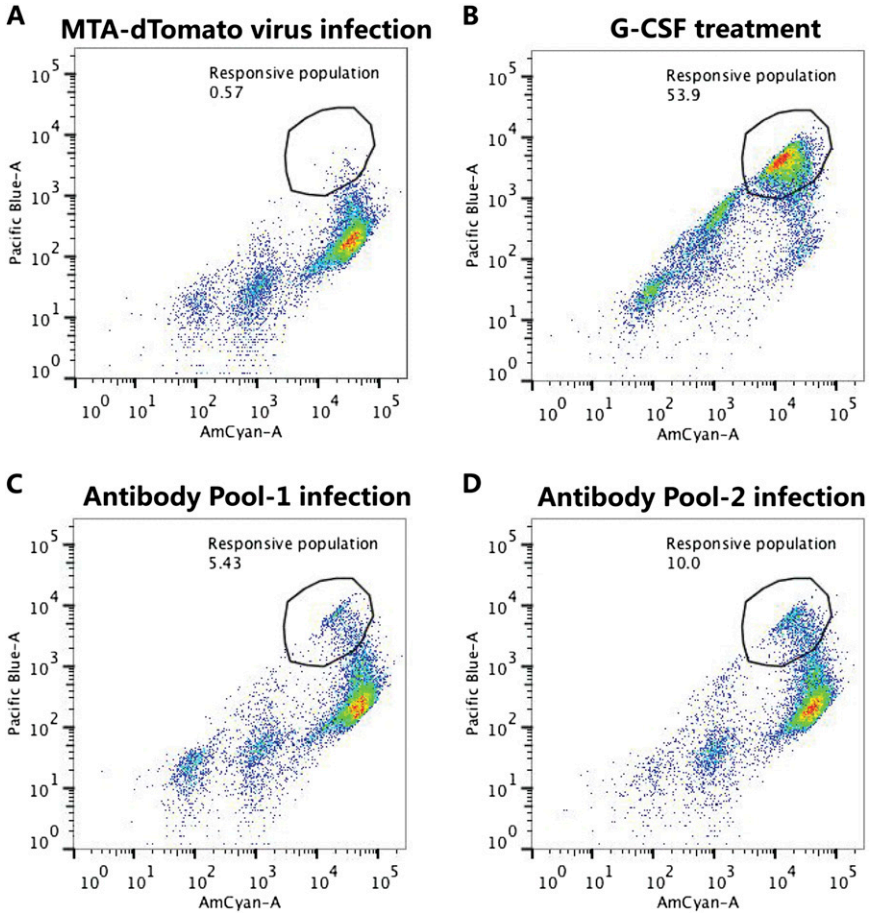


Fig. S3. The preselected plasma membrane tethered antibody libraries activate the reporter cell line. (*A*) As a control, SIE/BLA/SIG cells were infected by lentiviruses expressing membrane tethered Tomato red florescence protein (MTA-Tomato) followed by incubation with the FRET substrate (CCF4-AM). These control cells showed little activation. (*B*) Treatment with 1 ng/mL G-CSF activates the majority of the cells. (*C*) The reporter cells were infected by the lentivirus antibody library that had been enriched by panning against the G-CSFR ectodomain. (*D*) The activated pool of cells from *C* was collected and the antibody genes were recovered. The genes were converted into a secondary MTA-antibody lentivirus library, and the reporter cells were infected again. In this second round a larger percentage of cells was induced. The same gate was applied to the four panels. The induced cells are indicated by a circle to underscore the differences between various treatments.

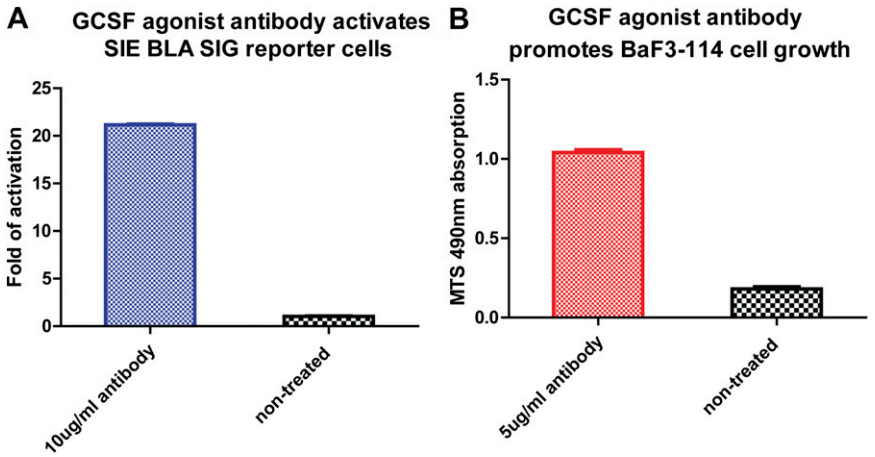


Fig. S4. The isolated soluble G-CSFR agonist antibody is capable of activating reporter cell lines. (*A*) The SIE/BLA/SIG cells were activated after the agonist antibody treatment. β-lactamase activity is measured by the FRET signal from hydrolysis of the CCF4-AM substrate. (*B*) The growth of the Ba/F3–G-CSFR cell line (BaF3-114) is stimulated by 2 d of treatment with the agonist antibody as measured by the MTS assay.

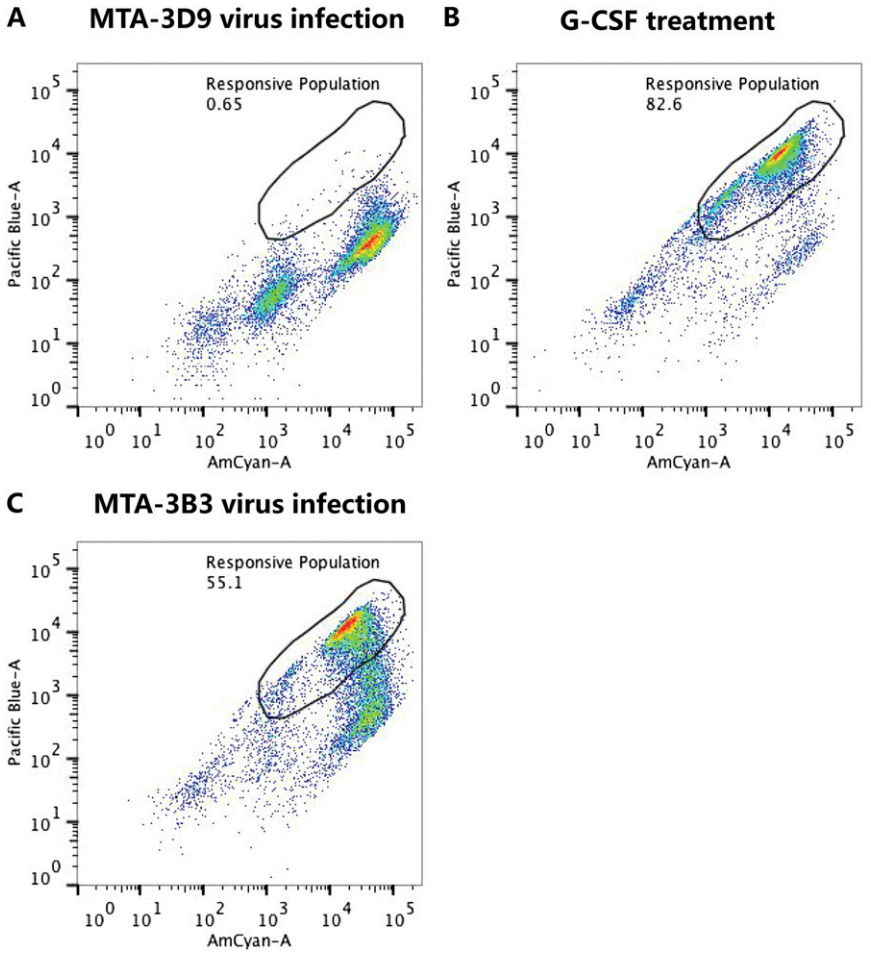


Fig. S5. The membrane tethered agonist antibody activated the G-CSFR reporter cells. (*A*) As a control, it was shown that the membrane tethered TPOR agonist antibody (MTA-3D9) does not activate the G-CSFR reporter cells. (*B*) G-CSF (1 ng/mL) treatment potently activates the reporter cells. (*C*) Lentivirus mediated delivery of the membrane-tethered agonist antibody activates the SIE/BLA/SIG cells.

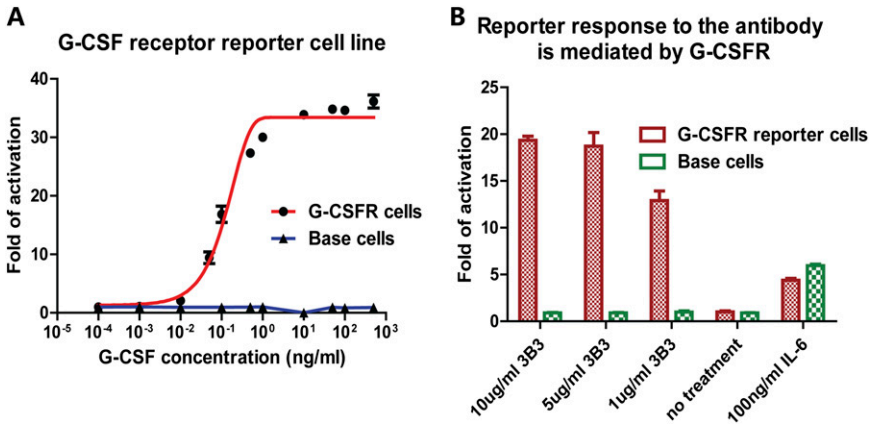


Fig. S6. The response of the SIE/BLA/SIG cells are strictly receptor dependent. (*A*) G-CSF activates the reporter cell line in a dose-dependent manner (round dots/red fitted curve), whereas the base cells (SIE/BLA cells used for constructing the reporter cell line) that did not contain the G-CSFR did not respond to any dose of G-CSF (triangles/blue fitted curve). (*B*) The reporter cell line responds to the agonist antibody (3B3) in a dose dependent manner (red column), whereas the base cells have no response to any dose of 3B3 (green column). Both cell lines respond to treatment with IL-6.

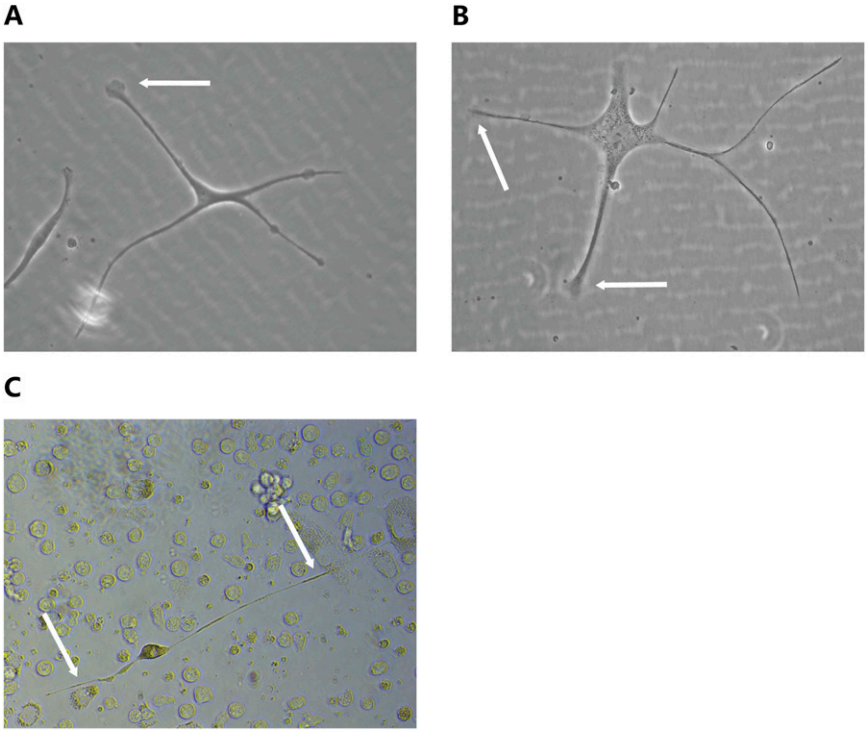


Fig. S7. Typical morphology of the antibody induced cells. (*A* and *B*) Single cells that are attached to glass coverslips after 2 wk of treatment with the G-SCF agonist antibody. White arrows point to growth cones. (*C*) "Bi-polar" morphology of some cells after 10 d in culture. Arrows indicate long neuritis.

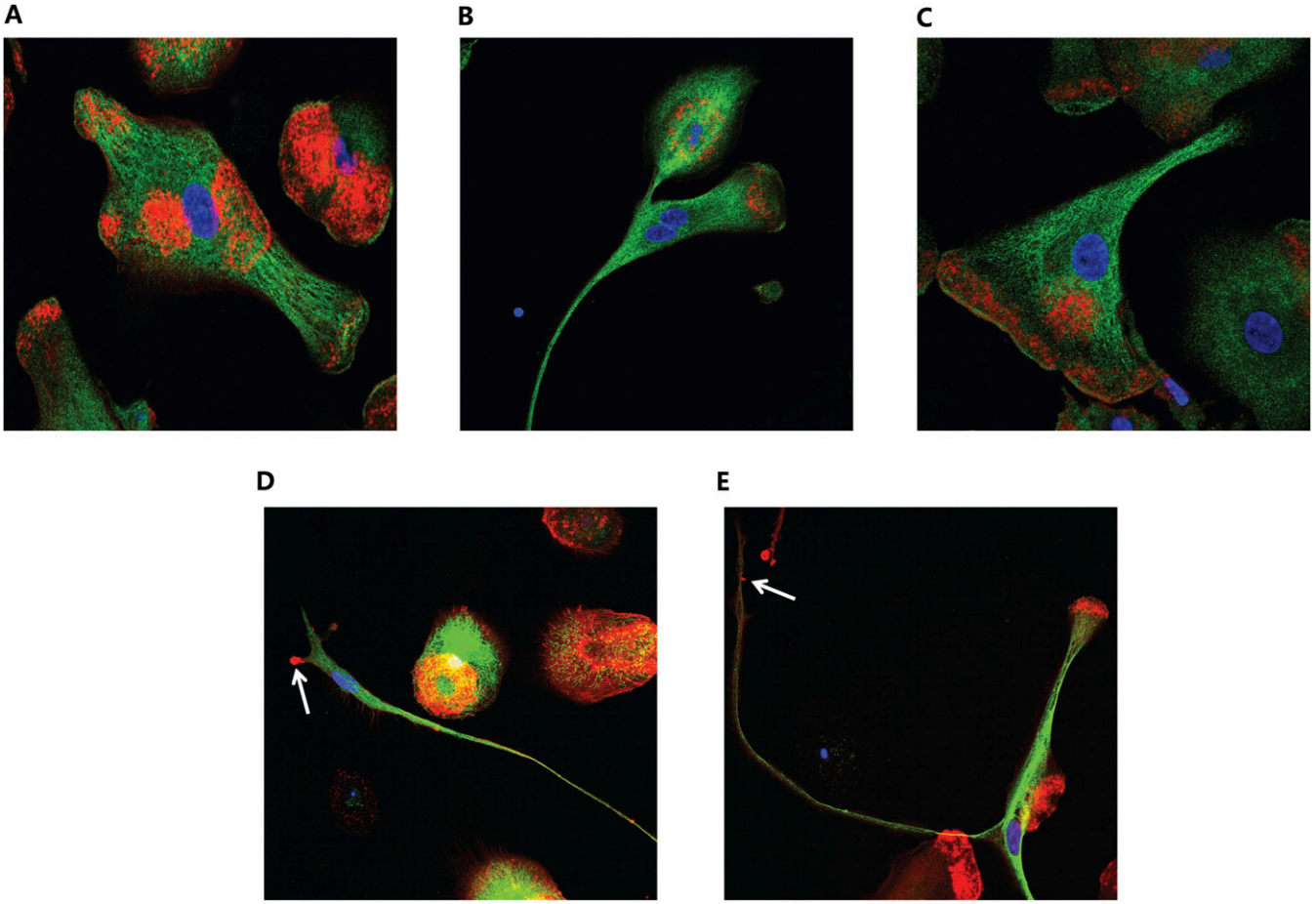
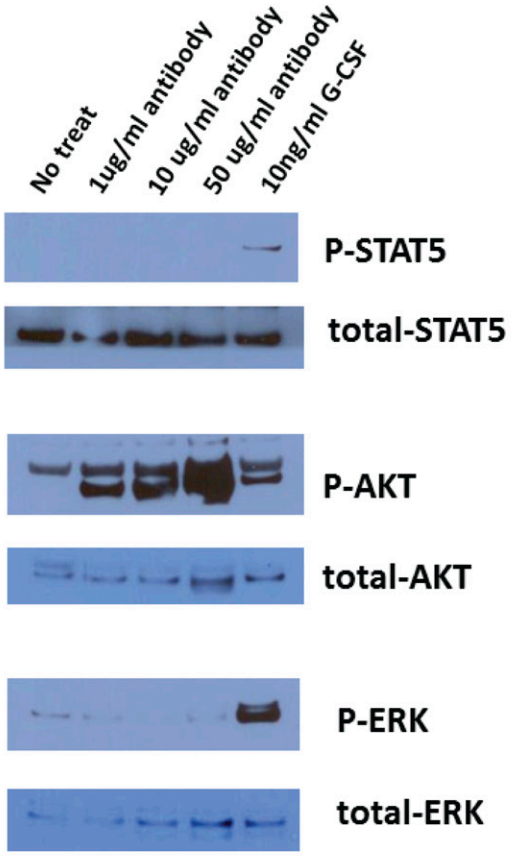


Fig. S8. (*A*–*C*) Nestin staining of the attached cells. Cells were cultured for 2 wk in the presence of the 3B3 antibody, fixed, and stained for Nestin (green). Actin is labeled by Phalloidin-eFluor 570 (red). (*D* and *E*). Extensive neurite length highlighted after Tuj-1 staining (green). Areas enriched for actin are red. White arrows highlight the "spine-like" bodies.

A



CD34 signaling change upon treatment.

B

Up-Down Regulation (comparing to control group)			
Symbol	G-CSF treated	Antibody treated	Description
TNR	-17.7987	-12.9103	Tenascin R (restrictin, janusin)
NEUROG1	1.344	-2.8421	Neurogenin 1
PTN	2.7045	-2.2055	Pleiotrophin
ASCL1	1.2054	-1.7391	Achaete-scute complex homolog 1 (Drosophila)
DCX	-2.3975	-1.7391	Doublecortin
GDNF	20.8234	-1.7391	Glial cell derived neurotrophic factor
NEUROD1	-2.3975	-1.7391	Neurogenic differentiation 1
NTF3	-2.3975	-1.7391	Neurotrophin 3
NTN1	1.1248	-1.2383	Netrin 1
HEY2	4.0525	-1.1569	Hairy/enhancer-of-split related with YRPW motif 2
BMP4	1.686	1.0239	Bone morphogenetic protein 4
PAX5	19.6514	1.1347	Paired box 5
DRD2	-1.1248	1.2383	Dopamine receptor D2
NOG	5.631	1.3017	Noggin
NRG1	1.7763	1.3452	Neuregulin 1
BCL2	1.3264	1.4982	B-cell CLL/lymphoma 2
NR2E3	2.2133	1.6406	Nuclear receptor subfamily 2, group E, member 3
SHH	2.3488	1.6484	Sonic hedgehog
POU4F1	2.2664	1.9088	POU class 4 homeobox 1
ACHE	-1.2539	2.0301	Acetylcholinesterase
HDAC4	3.1866	2.3083	Histone deacetylase 4
CHRM2	5.4691	2.3222	Cholinergic receptor, muscarinic 2
ALK	1.4857	2.5424	Anaplastic lymphoma receptor tyrosine kinase
ARTN	5.7668	2.5693	Artemin
PARD3	1.8311	2.5879	Par-3 partitioning defective 3 homolog (C. elegans)
BMPR1B	3.6934	2.6752	Bone morphogenetic protein 1b
FGF2	4.9774	2.7429	Fibroblast growth factor 2 (basic)
EP300	2.5375	2.8274	E1A binding protein p300
GRIN1	1.1458	3.0473	Glutamate receptor, ionotropic, N-methyl D-aspartate 1
DVL3	2.0741	3.1654	Dishevelled, dsh homolog 3 (Drosophila)
CDK5R1	3.3546	3.4993	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)
NEUROG2	1.3973	3.5693	Neurogenin 2
BDNF	11.7857	3.6076	Brain-derived neurotrophic factor
VEGFA	3.9145	3.7064	Vascular endothelial growth factor A
HEYL	3.1789	3.7446	Hairy/enhancer-of-split related with YRPW motif-like
TGFB1	2.7784	3.8124	Transforming growth factor, beta 1
BMP2	2.1519	4.0252	Bone morphogenetic protein 2
HEY1	-1.2668	4.1308	Hairy/enhancer-of-split related with YRPW motif 1
GAPDH	3.8356	4.1493	Glyceraldehyde-3-phosphate dehydrogenase
MLL	4.9881	4.3751	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)
HES1	1.5399	4.3993	Hairy and enhancer of split 1, (Drosophila)
ROBO1	2.2841	4.8885	Roundabout, axon guidance receptor, homolog 1 (Drosophila)
DLG4	3.3995	4.9524	Discs, large homolog 4 (Drosophila)
APBB1	2.9433	5.017	Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)
NF1	4.3342	5.4716	Neurofibromin 1
HPRT1	4.5513	5.4894	Hypoxanthine phosphoribosyltransferase 1
PAX3	4.1448	5.9948	Paired box 3
ERBB2	5.6796	6.1246	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
SOX8	-1.9254	6.1456	SRY (sex determining region Y)-box 8
POU3F3	-1.794	6.4601	POU class 3 homeobox 3
EGF	21.1167	6.7184	Epidermal growth factor
MEF2C	7.3553	6.8019	Myocyte enhancer factor 2C
FLNA	4.2498	7.5218	Filamin A, alpha
PAFAH1B1	5.67	7.54	Platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)
S100A6	4.4917	7.6968	S100 calcium binding protein A6
NOTCH1	5.3877	8.5092	Notch 1
SLIT2	1.3759	8.5862	Slit homolog 2 (Drosophila)
EFNB1	5.1437	8.6985	Ephrin-B1
SOD1	6.5288	9.1239	Superoxide dismutase 1, soluble
GPI	9.7885	9.1692	Glucose-6-phosphate isomerase
PAX6	8.9343	9.6074	Paired box 6
NOTCH2	5.9311	12.0427	Notch 2
CREB1	10.3732	12.2692	CAMP responsive element binding protein 1
APP	11.7066	12.7713	Amyloid beta (A4) precursor protein
NRP2	2.2078	14.6075	Neuropilin 2
S100B	2.0459	14.7618	S100 calcium binding protein B
MDK	28.2914	16.514	Midkine (neurite growth-promoting factor 2)
RPLP0	17.0713	16.7011	Ribosomal protein, large, P0
RAC1	8.8038	16.8501	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
STAT3	12.4851	17.2221	Signal transducer and activator of transcription 3 (acute-phase response factor)
SOX2	6.9825	17.3670	SRY (sex determining region Y)-box 2
CXCL1	8.3572	18.07	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
CDK5RAP2	4.1843	18.5043	CDK5 regulatory subunit associated protein 2
ADORA2A	11.1094	20.1578	Adenosine A2a receptor
B2M	14.186	20.5516	Beta-2-microglobulin
ACTB	16.1094	24.9335	Actin, beta
OLIG2	3.388	29.3751	Oligodendrocyte lineage transcription factor 2
IL3	5.3152	30.1039	Interleukin 3 (colony-stimulating factor, multiple)
RTN4	17.8768	32.8616	Reticulon 4
NDN	16.5192	33.8451	Necdin homolog (mouse)
NRP1	6.0134	34.2296	Neuropilin 1
ODZ1	41.759	35.5556	Odz, odd Oz/ten-m homolog 1 (Drosophila)
DLL1	3.3423	43.2457	Delta-like 1 (Drosophila)
TH	22.0316	47.2974	Tyrosine hydroxylase
MAP2	52.128	55.3154	Microtubule-associated protein 2
APOE	-1.255	57.7629	Apolipoprotein E
NRCAM	2.186	86.8107	Neuronal cell adhesion molecule
NDP	3.1484	229.5542	Norrie disease (pseudoglioma)
ADORA1	8.8274	314.9783	Adenosine A1 receptor

Fig. S9. (*A*) G-CSFR downstream signaling differs depending on the activators. CD34+ cells were treated with either the agonist antibody or G-CSF and the lysates were analyzed by Western blots using phosphorylation specific antibodies. (*B*) Quantitative PCR (qPCR)-based array analysis of the expression of 84 neurogenesis-related genes. The gene expression in control untreated CD34+ bone marrow cells was compared with either G-CSF or antibody treated cells after 2 wk in culture. All cells were from the same harvest and were simply divided into three parts. After culture, the RNA was extracted and cDNA was prepared. The cDNA was added to an array of 84 wells, each of which contained a different set of PCR primers for a gene related to neurogenesis. qPCR was carried out on the BioRad CFX-96 machine, and the data were analyzed by a web-based tool on SABioscience.com. An equal threshold was set to allow accurate comparison across samples, and the data were normalized to wells having the least variation according to the manufacturer's instructions.