Supporting Information

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SI Methods

Generation of Lentivectors Expressing Erythropoietin Receptor. A DNA sequence encoding mouse erythropoietin receptor (EPOR) was amplified by PCR from mouse thymic cDNA using Platinum *Pfx* DNA Polymerase (Promega.) at 1 mM $MgSO_4$. The forward primer 5′ATGGACAAACTCAGGGTG and the reverse primer 5′CTAGGAGCAGGCCACATA were used, and 40 amplification cycles of 94 °C for 15 s, 60 °C for 30 s, and 68 °C for 1 min 45 s were completed. The PCR product was treated with Taq polymerase (Promega) before subcloning in pCR2.1 (Invitrogen). A DNA fragment encoding EPOR for subcloning in a lentivector was amplified by PCR from one of the resulting clones. In the PCR product, the coding sequence was flanked at the 5′ end with a SpeI restriction site, which was copied from the multiple cloning sequence of pCR2.1 because the M13 reverse primer was used as a forward primer. At the 3′ end of the coding sequence, the BstBI site (underlined) was incorporated by the reverse primer 5′CGTAGCTTCGAAGGAGCAGGCCACATAGC to facilitate subcloning in frame with the V5 epitope. Platinum *Pfx* DNA Polymerase (Promega) was used at 1 mM $MgSO_4$, and 35 amplification cycles of 94 °C for 15 s, 50 °C for 30 s, and 68 °C for 1 min 45 s were completed. The PCR product was subcloned, between SpeI and BstBI sites, in the constitutive self-inactivating lentivector with the spleen focus-forming virus promoter (1) modified by us to include the V5 epitope, the mouse encephalomyocarditis internal ribosome entry site, and EGFP (2). Correct sequences of inserts were confirmed by DNA sequence analysis at the Genome Centre, Queen Mary University of London. Production of lentivector particles, gene transfer, and cloning of B104 cells were performed as described previously (2).

EPOR Expression and Activation in B104-EPOR Cells. The expression of EPOR in transduced cells was detected as described for other V5-tagged recombinant proteins (2). Briefly, cells were lysed with the buffer containing 10 mM Tris (pH 7.4), 100 mM NaCl, 50 mM NaF, 0.1% SDS, 1% Triton-X 100 (all from VWR), 1 mM EDTA, 1 mM EGTA, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 0.5% sodium deoxycholate, 10% glycerol, and 1 mM PMSF (all from Sigma), and the protease inhibitor mixture (Calbiochem). Cell lysates were separated by PAGE in the presence of 5% β-mercaptoethanol. EPOR was detected by immunoblotting with the anti-V5 mouse monoclonal antibody (Invitrogen).

To determine EPOR activation in response to erythropoietin (EPO), EPOR-expressing B104 cells were cultured until 90% confluent in six-well plates, serum-starved for 4 h, and stimulated or mock-stimulated with 50 ng/mL of human recombinant EPO (Peprotec) for 15 or 30 min. Lysates of control and stimulated cells were immunoprecipitated with rabbit polyclonal anti-EPOR antibody (Santa Cruz Biotechnology) and immunoblotted with a mixture of the phosphotyrosine-specific mouse mAbs pY20 (Abcam) and 4G10 (Millipore), 1 mg/mL each (anti-pY). After stripping at low pH, the blots were reprobed with anti-V5. Immunoblots were developed as described (2) using HRP-conjugated $F(ab')_2$ fragment of rabbit anti-mouse IgG (Zymed Laboratories) as a secondary antibody.

1. Demaison C, et al. (2002) High-level transduction and gene expression in hematopoietic repopulating cells using a human immunodeficiency virus type 1-based lentiviral vector containing an internal spleen focus forming virus promoter. *Hum Gene Ther* 13:803–813.

2. Annenkov A, et al. (2011) A chimeric receptor of the insulin-like growth factor receptor type 1 (IGFR1) and a single chain antibody specific to myelin oligodendrocyte glycoprotein activates the IGF1R signalling cascade in CG4 oligodendrocyte progenitors. *Biochim Biophys Acta* 1813:1428–1437.

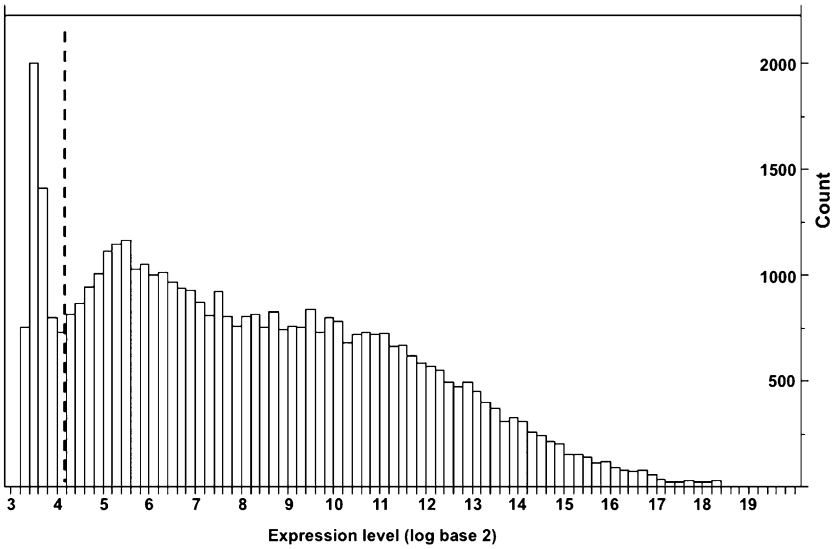

Fig. S1. Frequency distribution of expression data. The dashed vertical line shows the arbitrary threshold of 4.2 used to filter out low-expression transcripts.

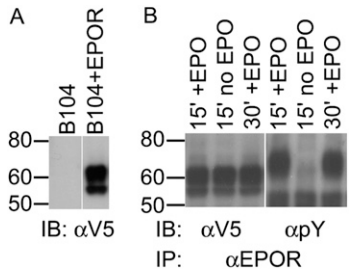

Fig. S2. Expression and activation of EPOR in B104 cells. (*A*) Untransduced B104 cells and the clone of EPOR-transduced B104 cells were lysed, and EPOR expression in cell lysates was determined by immunoblotting with anti-V5. (*B*) The clone of EPOR-expressing B104 cells was treated with EPO (50 ng/mL) for 15 or 30 min or was mock-stimulated for 15 min. EPOR was immunoprecipitated (IP) from cell lysates with anti-EPOR, separated by PAGE, and immunoblotted (IB) with anti-pY. The membranes were stripped and reprobed with anti-V5.

Table S1. Transcripts significantly changed by EPO in ischemic cortex at 2 h and relative changes in ischemia versus sham

		Ischemia+EPO vs. ischemia		Ischemia vs. sham	
Gene symbol	Accession number	Fold change	*P* value	Fold change	*P* value
Up-regulated					
XM_226521*	XM_226521	1.46	0.0173	−1.31	0.0395
Olr792_predicted	NM_001000378	1.45	0.0037	−0.64	0.0038
BF565770*	BF565770	1.33	0.0378	ns	—
AI010145*	AI010145	1.15	0.0119	ns	—
TC533613*	TC533613	1.14	0.0438	−0.55	0.0493
AA891571*	AA891571	1.11	0.0324	−1.28	0.0144
CB762850*	CB762850	1.05	0.0392	−0.96	0.0488
Cyp2a2	NM_012693	1.04	0.0179	ns	—
AI230744*	AI230744	0.90	0.0395	ns	—
AI599572*	AI599572	0.72	0.0262	ns	—
Jak3	NM_012855	0.59	0.0278	ns	—
Down-regulated					
XM_226554*	XM_226554	−2.04	0.0012	1.44	0.0040
Olr529_predicted	NM_001000674	−1.56	0.0128	ns	—
AI231805*	AI231805	−1.53	0.0054	ns	—
Olr153_predicted	NM_001000167	−1.50	0.0184	ns	—
BE107503*	BE107503	−1.38	0.0253	ns	—
LOC683790	XM_001067479	−1.33	0.0008	0.92	0.0027
RGD1560913_predicted	XM_574627	−1.29	0.0206	1.32	0.0107
BE117594*	BE117594	−1.14	0.0435	ns	—
BQ201357*	BQ201357	−1.03	0.0447	ns	—
AA818700*	AA818700	−0.93	0.0165	ns	—
AA858950*	AA858950	−0.92	0.0380	ns	—
Aqp6	NM_022181	−0.92	0.0238	ns	—
AI113235*	AI113235	−0.89	0.0345	ns	—
LOC684560	XM_001070979	−0.87	0.0352	ns	—
RGD1559517_predicted	XM_227127	−0.86	0.0366	0.67	0.0392
BG672648*	BG672648	−0.85	0.0308	ns	—
A_44_P630635*	A_44_P630635	−0.85	0.0270	ns	—
BE105878*	BE105878	−0.82	0.0441	1.08	0.0019
ENSRNOT00000038661	ENSRNOT00000038661	−0.81	0.0341	ns	—
Bmpr2†	XM_217409	−0.79	0.0040	ns	—
BF567165	BF567165	−0.76	0.0273	ns	—
AA900594*	AA900594	−0.74	0.0136	ns	—
BE104341*	BE104341	−0.70	0.0424	ns	—
RGD1561261_predicted	XM_001053212	−0.70	0.0399	ns	—
Kcnd3†	NM_031739	−0.69	0.0465	ns	—
TC533561*	TC533561	−0.68	0.0014	ns	—
Igf1†	NM_178866	−0.67	0.0283	ns	—
TC562057*	TC562057	−0.67	0.0052	ns	—
Odz3_predicted†	XM_224841	−0.65	0.0473	ns	—
LOC685076†	XM_001062178	−0.63	0.0460	ns	—
TC558054*	TC558054	−0.62	0.0037	ns	—
BF545795*	BF545795	−0.61	0.0361	ns	—
BQ200021*	BQ200021	−0.59	0.0473	ns	—

Fold change is expressed as log2 ratio and is the average of triplicate samples. All transcripts changed more than 1.5-fold, $P < 0.05$ in ischemia+EPO vs. ischemia are included, and the relative changes in ischemia vs. sham are reported. ns, not significant.

*Unmapped probe IDs.

†Transcripts identified by different probes/replicates, of which only one probe/replicate changed significantly.

Table S2. Transcripts significantly changed by EPO in ischemic cortex at 6 h and relative changes in ischemia versus sham

		Ischemia+EPO vs. ischemia		Ischemia vs. sham	
Gene symbol	Accession number	Fold change	*P* value	Fold change	*P* value
Up-regulated					
RGD1304775_predicted	XM_237151	2.80	0.0469	ns	—
XM_344767*	XM_344767	2.76	0.0183	ns	—
XM_224708*	XM_224708	2.74	0.0429	ns	—
AA956764*	AA956764	2.60	0.0029	ns	—
CB547501*	CB547501	2.50	0.0129	ns	—
RGD1310265_predicted	XM_001070727	2.38	0.0423	ns	—
Krt14	D63774	2.32	0.0377	ns	—
Slc10a1	NM_017047	2.28	0.0273	ns	—
LOC679379	XM_001055377	2.23	0.0468	ns	—
XM_576553*	XM_576553	2.19	0.0035	ns	—
ENSRNOT00000014809	ENSRNOT00000014809	2.02	0.0229	ns	—
AI598863*	AI598863	1.96	0.0483	−1.25	0.0051
AI599332*	AI599332	1.93	0.0323	ns	—
Ces5	XM_341636	1.62	0.0479	ns	—
RGD1563378_predicted	XM_228994	1.60	0.0342	ns	—
Olr1461_predicted	NM_001000022	1.46	0.0476	ns	—
BE111679*	BE111679	1.33	0.0210	ns	—
BE101481*	BE101481	1.26	0.0251	ns	—
TC541788*	TC541788	1.22	0.0341	ns	—
BI288013*	BI288013	1.21	0.0287	ns	—
RGD1565310_predicted†	XM_343014	1.12	0.0064	ns	—
Bdnf	NM_012513	1.08	0.0323	1.49	0.0026
Bdnf	NM_012513	1.07	0.0450	1.47	0.0048
Bdnf	NM_012513	1.07	0.0390	1.58	0.0049
XM_342155*	XM_342155	1.05	0.0276	ns	—
Bdnf	NM_012513	1.05	0.0483	1.49	0.0040
Bdnf	NM_012513	1.02	0.0419	1.54	0.0033
Bdnf	NM_012513	1.02	0.0405	1.50	0.0023
TC531175*	TC531175	1.02	0.0290	−1.06	0.0119
AW142999*	AW142999	1.01	0.0400	2.38	0.0007
Dusp5	NM_133578	1.01	0.0333	1.42	0.0018
AW142932*	AW142932	1.01	0.0408	2.35	0.0003
Egr2	NM_053633	1.01	0.0077	1.42	0.0089
Olr372_predicted	NM_001001048	1.00	0.0153	ns	—
TC530277*	TC530277	0.89	0.0071	0.86	0.0180
Arc	NM_019361	0.88	0.0433	1.57	0.0008
Arc	NM_019361	0.87	0.0475	1.60	0.0003
Arc	NM_019361	0.86	0.0461	1.59	0.0004
Arc	NM_019361	0.86	0.0439	1.60	0.0006
Fosl2	NM_012954	0.86	0.0099	ns	—
Mas1	NM_012757	0.85	0.0188	ns	—
Arc	NM_019361	0.85	0.0439	1.59	0.0005
Egr4	NM_019137	0.85	0.0253	1.08	0.0066
Arc	NM_019361	0.85	0.0459	1.58	0.0001
LOC684624	XM_001070871	0.84	0.0472	ns	—
Arc	NM_019361	0.84	0.0396	1.61	0.0003
BF281861*	BF281861	0.80	0.0299	ns	
AABR03057977*	AABR03057977	0.80	0.0449	−0.87	0.0438
TC532746*	TC532746	0.77	0.0407	−1.44	0.0045
TC542653*	TC542653	0.77	0.0003	ns	—
RGD1565022_predicted†	XM_216007	0.76	0.0287	ns	—
TC547308*	TC547308	0.76	0.0496	ns	—
Rem2	NM_022685	0.75	0.0183	1.29	0.0192
AF050661*	AF050661	0.74	0.0268	ns	—
Olr1678_predicted	NM_001000893	0.73	0.0395	ns	—
TC549515*	TC549515	0.72	0.0319	ns	—
Prssl1	NM_001003956	0.68	0.0057	ns	—
Nr4a3	NM_031628	0.68	0.0152	ns	—
AI137349*	AI137349	0.67	0.0406	ns	—
XM_224859	XM_224859	0.66	0.0169	ns	—

Table S2. Cont.

Gene symbol	Accession number	Ischemia+EPO vs. ischemia		Ischemia vs. sham	
		Fold change	*P* value	Fold change	*P* value
TC545861*	TC545861	0.65	0.0175	−0.69	0.0318
Numb†	DQ336705	0.65	0.0409	ns	—
XM_344386*	XM_344386	0.65	0.0040	−1.02	0.0072
TC549896*	TC549896	0.65	0.0233	−0.84	0.0182
RGD1311223_predicted	XM_345971	0.65	0.0300	ns	—
TC543180*	TC543180	0.65	0.0373	−0.73	0.0220
TC522684*	TC522684	0.64	0.0209	−0.91	0.0018
Nr4a3	DQ268830	0.64	0.0040	ns	—
Egr1	NM_012551	0.63	0.0326	ns	—
TC563722*	TC563722	0.63	0.0340	ns	—
Egr1	NM_012551	0.62	0.0239	ns	—
Ccl7	NM_001007612	0.62	0.0195	2.65	0.0064
Egr1	NM_012551	0.62	0.0225	ns	—
Cdkl3	NM_021772	0.61	0.0274	ns	—
Egr1	NM_012551	0.61	0.0190	ns	—
RGD1564664_predicted†	XM_575179	0.60	0.0472	1.34	0.0007
RGD1562685_predicted	XM_231463	0.60	0.0099	ns	—
Egr1	NM_012551	0.60	0.0267	ns	—
Egr1	NM_012551	0.60	0.0382	ns	—
AI710695*	AI710695	0.60	0.0491	ns	—
AA875032*	AA875032	0.59	0.0226	0.67	0.0057
Egr1	NM_012551	0.59	0.0326	ns	—
Angptl4	NM_199115	0.59	0.0285	1.02	0.0123
TC567892*	TC567892	0.59	0.0466	ns	—
Ccl7	NM_001007612	0.58	0.0298	2.84	0.0008
Egr1	NM_012551	0.58	0.0341	ns	—
Down-regulated					
Olr750_predicted	NM_001000366	−1.56	0.0321	ns	—
Atp7a	NM_052803	−1.21	0.0159	ns	—
Trem1_predicted	XM_217336	−1.03	0.0243	1.67	0.0057
Olr1630_predicted	NM_001000092	−0.97	0.0240	ns	—
BM386916*	BM386916	−0.96	0.0173	0.64	0.0139
BM384709*	BM384709	−0.95	0.0168	ns	—
TC542144*	TC542144	−0.94	0.0194	ns	—
RGD1307937	NM_001013877	−0.82	0.0265	ns	—
AI385136*	AI385136	−0.79	0.0137	ns	—
BI277534*	BI277534	−0.78	0.0381	ns	—
RGD1310352	XM_220404	−0.71	0.0141	ns	—
Ar†	NM_012502	−0.71	0.0234	−1.13	0.0299
Alg5†	NM_001025407	−0.69	0.0074	ns	—
AA925183*	AA925183	−0.69	0.0046	ns	—
Zfp606	XM_218283	−0.67	0.0429	ns	—
Tex15_predicted†	XM_214365	−0.66	0.0258	ns	—
Cxcl2	NM_053647	−0.65	0.0369	6.49	1.4E-07
Cbll1_predicted†	XM_001073155	−0.61	0.0488	0.64	0.0053
LOC679115	XM_001054757	−0.60	0.0235	ns	—
Olr1271†	NM_173300	−0.60	0.0260	ns	—
RGD1310980_predicted	XM_343381	−0.60	0.0358	ns	—
LOC680443	XM_001057208	−0.60	0.0115	ns	—
Rnf24_predicted	XM_342522	−0.59	0.0243	ns	—
Crispld1_predicted	XM_237258	−0.59	0.0491	ns	—

Fold change is expressed as log2 ratio and is the average of triplicate samples. All transcripts changed more than 1.5-fold, $P < 0.05$ in ischemia+EPO vs. ischemia are included, and the relative changes in ischemia vs. sham are reported. The table includes replicates identifying the same genes. ns, not significant.
*Unmapped probe IDs.
†Transcripts identified by different probes/replicates, of which only one probe/replicate significantly changed. These transcripts were not included in Table 2.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)