

Supplementary Figure and Table Legends

Supplementary Figure 1. DNA methylation-based classification of parental

tumors. a. The parental tumors for 8/9 primary cultures used in this study were analyzed with 450k arrays for DNA methylation. Copy number variation (CNV) profiles are shown. **b.** Molecular subtype by DNA methylation and RNA-seq (where available), cytogenetic profile, important CNV information, IDH1 status and MGMT promoter methylation status are shown. NGS, next-generation sequencing.

Supplementary Figure 2. GPR133 expression information. a.

Representative flow cytometry analysis of 3 primary GBM cultures for CD133 and GPR133. **b.** Summary statistics describing the abundance of GPR133+ and CD133+ cells in 3 primary cultures based on flow cytometric quantitation. **c.** Analysis of GTEx data on human tissue-specific expression of *GPR133*. Normal brain tissue expresses very low amounts of *GPR133*.

Supplementary Figure 3. Expression pattern of GPR133 in human GBM

biospecimens and tumor xenografts. a. GPR133 immunoreactivity in hypoxic PPN regions of adjacent sections of an FFPE human GBM biospecimen, as detected with a

rabbit polyclonal (i) and a mouse monoclonal (ii) antibody. In (i), GPR133 expression overlaps with CD133 expression (arrowheads). In (ii), GPR133 and CA9 co-localize in PPN cells (arrowheads). **bi-iii.** GPR133 immunoreactivity co-localizes with pimonidazole in tumor xenografts in the mouse brain (n=3 animals). Evans Blue highlights the vasculature, which is relatively scant in the more hypoxic portion of the tumor staining positive for pimonidazole. The region of interest in (ii) is shown in high magnification in (iii), where several cells with overlapping GPR133 and pimonidazole staining are observed (arrowheads). N: necrosis; PPN: pseudopalisading necrosis.

Supplementary Figure 4. Characterization of a new mouse monoclonal anti-

GPR133 antibody. **a.** The antibody recognizes the pentraxin/concanavalin A domain of GPR133's N-terminal ectodomain. **b.** CHO-GPR133 and mock-transfected cells (CHO-Mock) were subjected to qRT-PCR analysis for *GPR133* mRNA with and without doxycycline (1 μ g/ml). **c.** In a quality control assay, CHO-Mock cells were immunostained with the anti-GPR133 mouse monoclonal antibody with and without doxycycline. Both permeabilized (i) and non-permeabilized (ii) preparations were used.

We did not observe any GPR133 immunostaining.

Supplementary Figure 5. Quantitation of *GPR133* mRNA with two different Taqman assays and the distribution of reads obtained with RNA-seq reveal that full-length *GPR133* is the predominant isoform in GBM. **a.** The relative amounts of *GPR133* mRNA detected with two different Taqman assays did not differ, suggesting that the full-length *GPR133* splice variant is the dominant one in 5 primary GBM cultures (n=3 replicates/condition; t-test for each culture, P>0.05 throughout). **b.** Sashimi plots showing the distribution of reads obtained from RNA-seq comparison of FACS-isolated CD133+ and CD133- cells from GBML8 sample (average of 2 RNA-seq experiments shown). Reads were aligned with predicted *Ensembl* transcripts to show exon usage.

Supplementary Figure 6. *In vitro* effects of *GPR133* knockdown in GBML8. The layout of this figure is identical to **Figure 4**, but describes data obtained with GBML8. **a.** **i.** Representative example of the effect of *GPR133* knockdown (*GPR133*-KD#1) on the number of *GPR133*+ cells, as shown by flow cytometry. **ii.** Cumulative statistics on the reduction of *GPR133*+ cells by *GPR133* knockdown (n=3 experiments, t-test, P<0.003). **b.** *GPR133* knockdown decreases *GPR133* mRNA levels (n=3 experiments, t-test, P<0.0001). **c.** Flow cytometry indicates reduction in the abundance of CD133+ cells

after GPR133 knockdown. A representative example (i) and cumulative statistics (ii) (n=3 experiments, t-test, $P < 0.0005$) are shown. **d,e.** Knockdown of GPR133 reduces the percentage of Ki67+ cells in both normoxia (n=3 experiments, t-test, $P < 0.02$) and hypoxia (n=3 experiments, t-test, $P < 0.05$). **e.** GPR133 knockdown impairs tumorsphere formation in (i) normoxia (n=3 experiments, t-test, $P < 0.002$) and (ii) hypoxia (n=3 experiments, t-test, $P < 0.002$).

Supplementary Figure 7. *In vitro* effects of GPR133 knockdown on cell proliferation. We tested the effects of GPR133 knockdown (GPR133-KD#1) on cell proliferation using immunostaining for phospho-H3 (pH3) in GBML8 (**a**) and GBML20 (**b**) cells *in vitro*. Representative images are shown in (**ai**) and (**bi**). Summary statistics in (**aii**) and (**bii**) indicate that GPR133 knockdown produces a significant reduction in pH3 staining, indicating reduced tumor cell proliferation (GBML8: n=3 experiments, t-test, $P < 0.001$; GBML20: n=3 experiments, t-test, $P < 0.0001$).

Supplementary Figure 8. Effects of GPR133 knockdown on limiting dilution assays and sphere size in GBML8 and GBML20. **a,b.** Dissociated GBML8 (**a**) and GBML20 (**b**) cells bearing either scrambled or GPR133-KD#1 constructs were plated in

limiting dilutions (1,10,100 and 1000 cells/well) and allowed to form spheres in either (i) normoxia or (ii) hypoxia. GPR133-KD in all conditions led to a significant reduction in the probability of sphere formation, indicating a reduction in stem cell frequency ($P < 0.0001$). Along these lines, the number of spheres formed/well was significantly lower in (i) normoxia (GBML8: ANOVA $F_{(1,12)} = 47.68$, $P < 0.0001$; and GBML20: ANOVA $F_{(1,12)} = 84.55$, $P < 0.0001$) and (ii) hypoxia (GBML8: ANOVA $F_{(1,12)} = 40.59$, $P < 0.0001$; and GBML20: ANOVA $F_{(1,12)} = 44.57$, $P < 0.0001$). P values for *post hoc* multiple comparisons by Tukey's test are shown in the figure ($n = 5$ wells/condition). **c.** Analysis of the size of the spheres formed using (i) GBML8 and (ii). GBML20 cells did not reveal any significant difference.

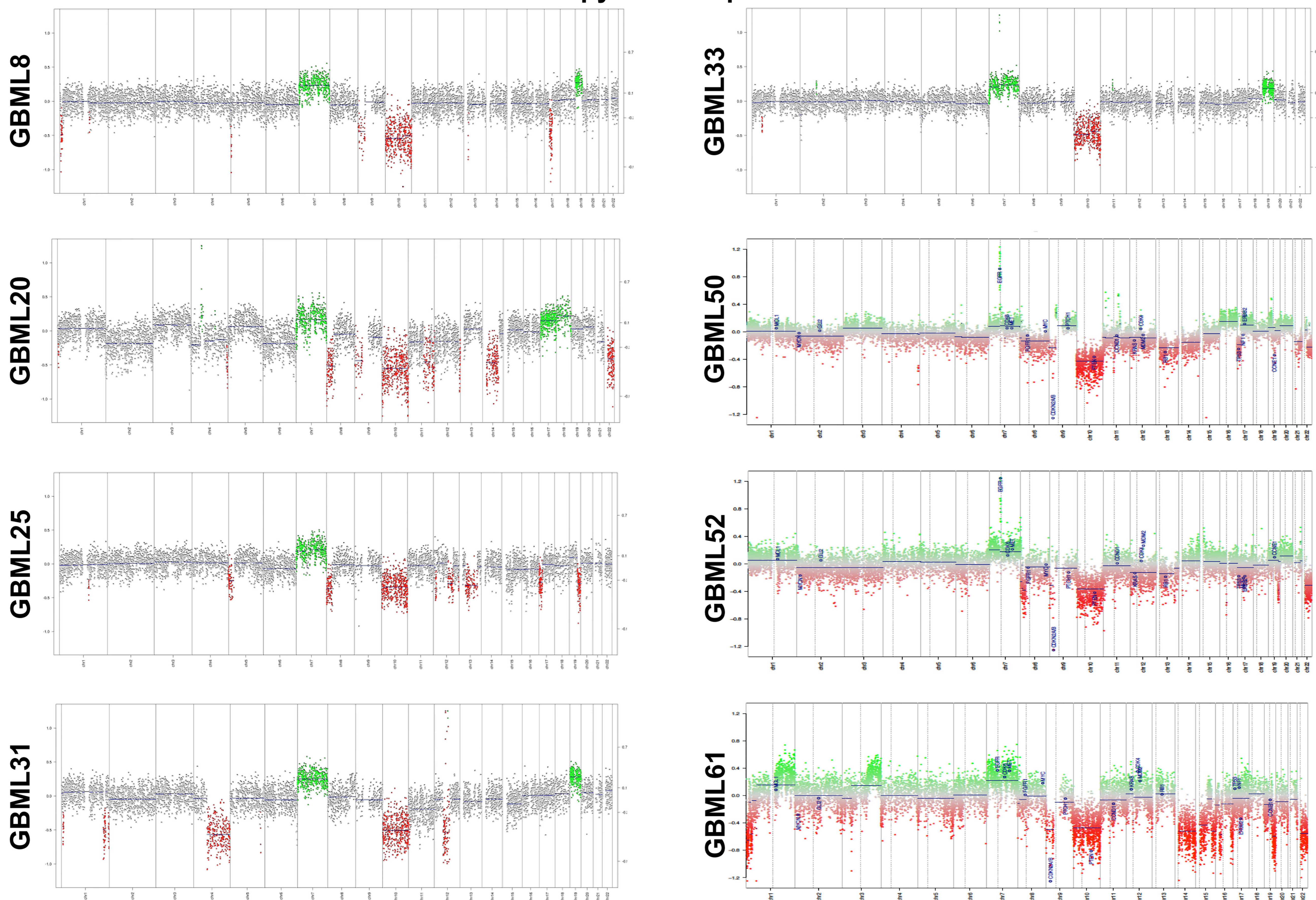
Supplementary Table 1. a. Genes upregulated in CD133+ vs. CD133- GBM cells. **b.** Genes downregulated in CD133+ vs. CD133- GBM cells.

Supplementary Table 2. a. Taqman Assays, **b.** PCR primers and **c.** shRNA constructs used in the study.

Supplementary Table 3. Antibodies used for immunofluorescence staining.

a

Copy number profiles



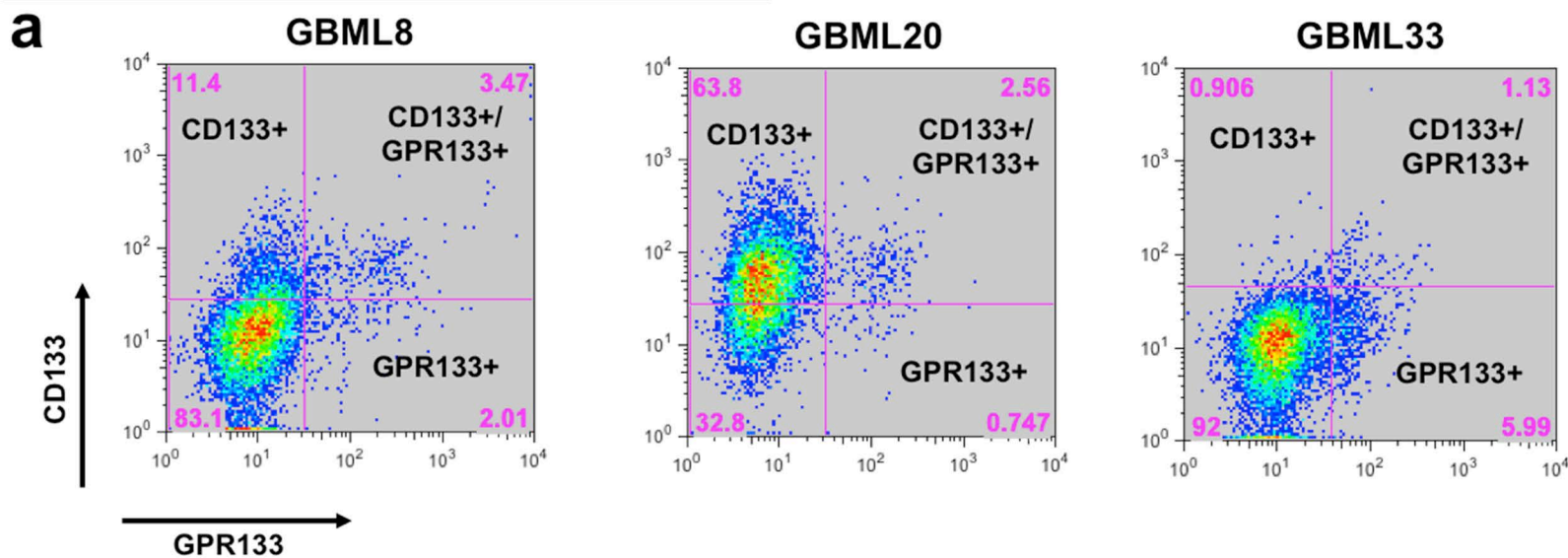
Chr 1-22 →

Chr 1-22 →

b

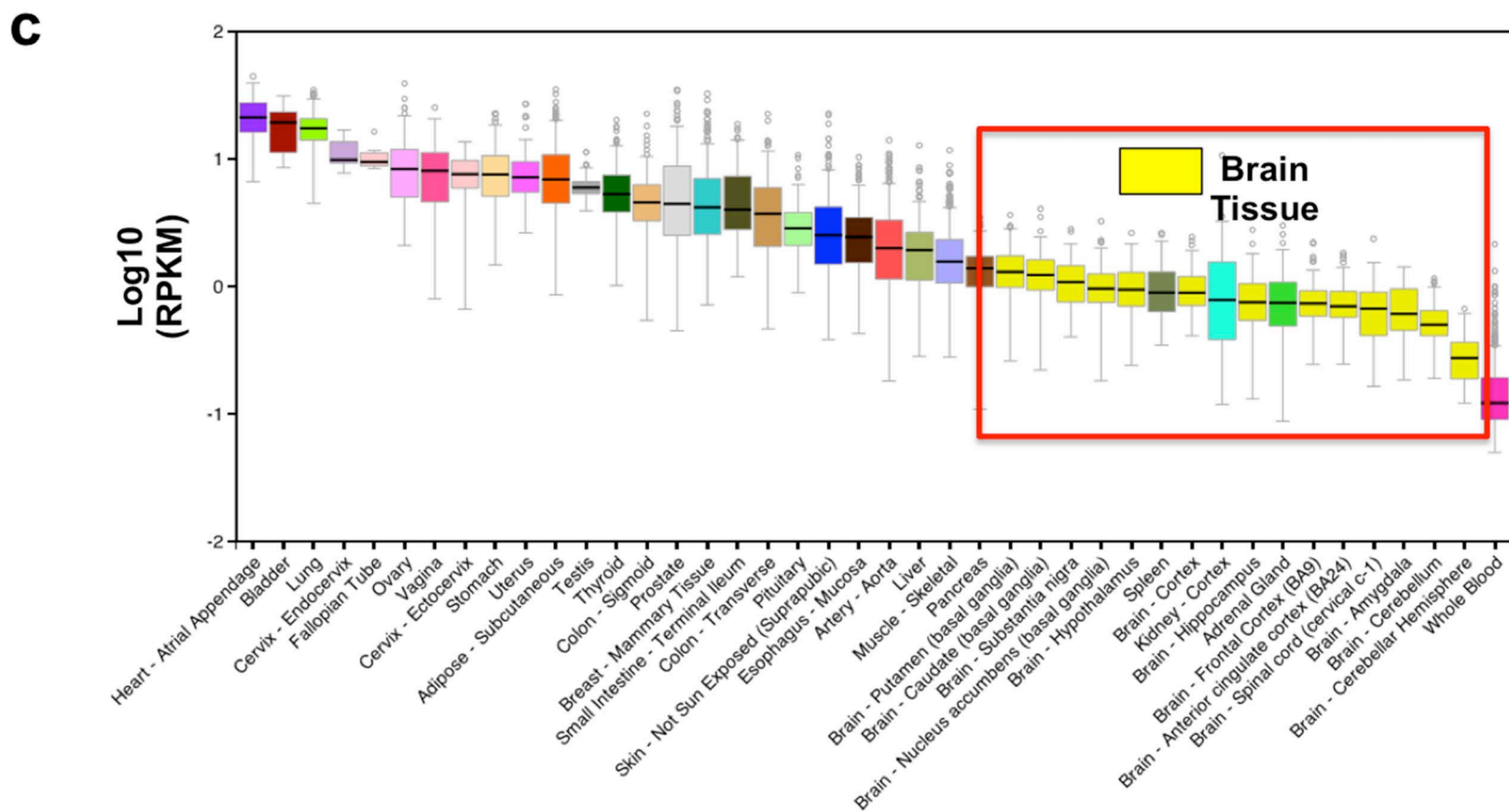
Patient sample	Score/subtype by DNA Methylation array	Score/subtype by RNA-seq	Cytogenetics	Other implied changes from CNV or NGS analysis	IDH1 status	MGMT methylation
GBML8	Mesenchymal	Mesenchymal	7+, 9p-, 10-, 17q-, 19p+	CDKN2A loss, PTEN loss, Nf1 loss	IDH wild-type	Methylated
GBML20	GBM RTK1 (proneural)	Proneural	7+, 8p-, 10-, 11q-, 14-, 17+	PDGFRA amp, PTEN loss	IDH wild-type	Not methylated
GBML25	GBM Classic/Mesenchymal & GBM RTK1	Not available	4q-, 7+, 10-, 12q-, 12q+, 19+	PTEN loss, MDM2 amp	IDH wild-type	Not methylated
GBML31	GBM Classic	Not available	7+, 10-, 19+	EGFR amp, PTEN loss	IDH wild-type	Methylated
GBML33	GBM RTK1 (proneural)	Not available	5p-, 7+, 8p-, 10-, 12q-, 13q-, 17p-, 19q-	TERT loss, PTEN loss, Rb1 loss, TP53 loss	IDH wild-type	Not methylated
GBML50	GBM RTK1 (proneural)	Not available	10-	EGFR amp, CDKN2A loss	IDH wild-type	Not methylated
GBML52	GBM Classic/Mesenchymal	Classic	7+, 8p-, 10-, 19q-, 22-	EGFR amp, CDKN2A loss, TP53 mutation, PTEN mutation	IDH wild-type	Not methylated
GBML61	GBM RTK1 (proneural)	Not available	1p-, 3q+, 7+, 9p-, 10-, 14-, 15q-, 19q-, 22-	CDKN2A loss, PTEN loss	IDH wild-type	Not methylated
GBML62	Not available	Not available	Not available	TP53 mutation, Rb1 mutation	IDH wild-type	Not methylated

Supplementary Figure 2



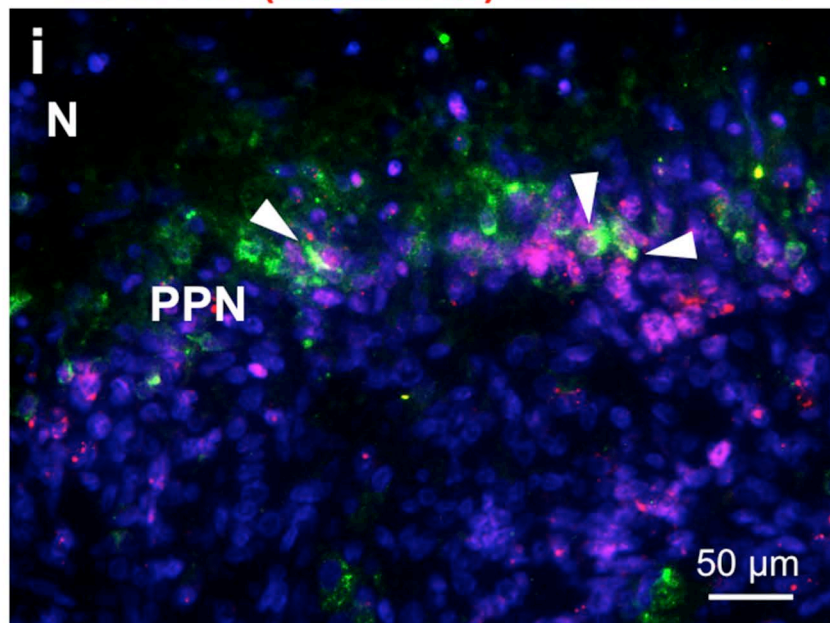
b

	% CD133+ cells	% GPR133+ cells	% GPR133+ cells in CD133+ population	% GPR133+ cells in CD133- population
GBML8	31.29 ± 16.00	2.95 ± 1.27	16.05 ± 4.78	1.79 ± 0.29
GBML20	71.13 ± 6.22	4.62 ± 0.86	4.92 ± 1.33	3.56 ± 1.09
GBML33	3.90 ± 2.51	9.39 ± 1.56	44.32 ± 13.32	6.84 ± 2.13
Cumulative (n=3 samples)	35.44 ± 19.52	5.65 ± 1.92	21.77 ± 11.72	4.06 ± 1.48

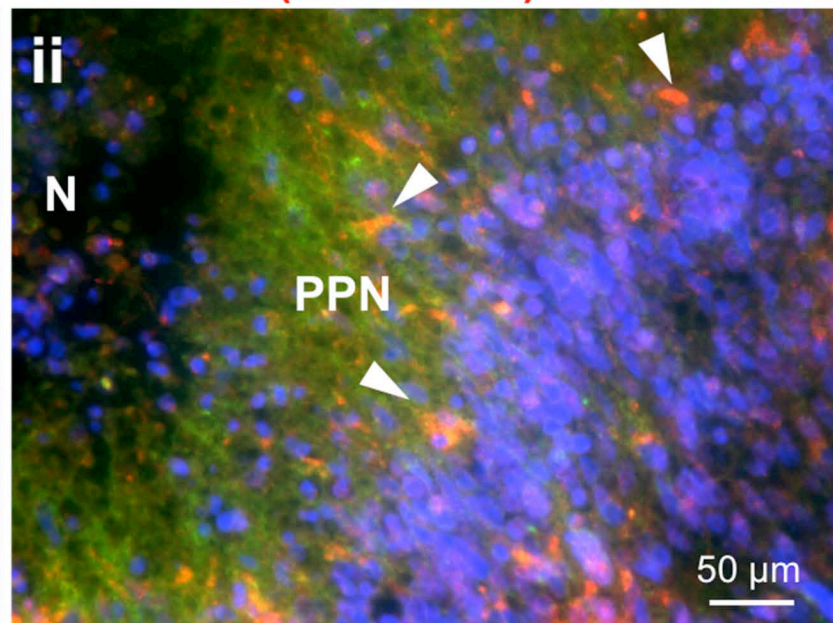


Supplementary Figure 3

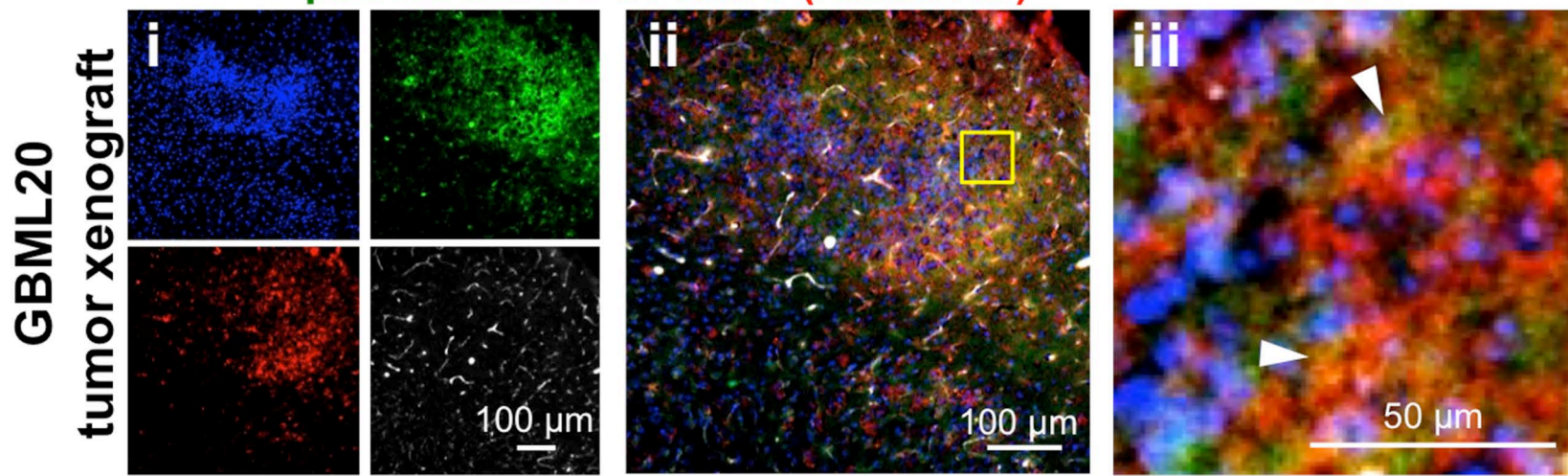
a GPR133 (rabbit Ab)/CD133/DAPI

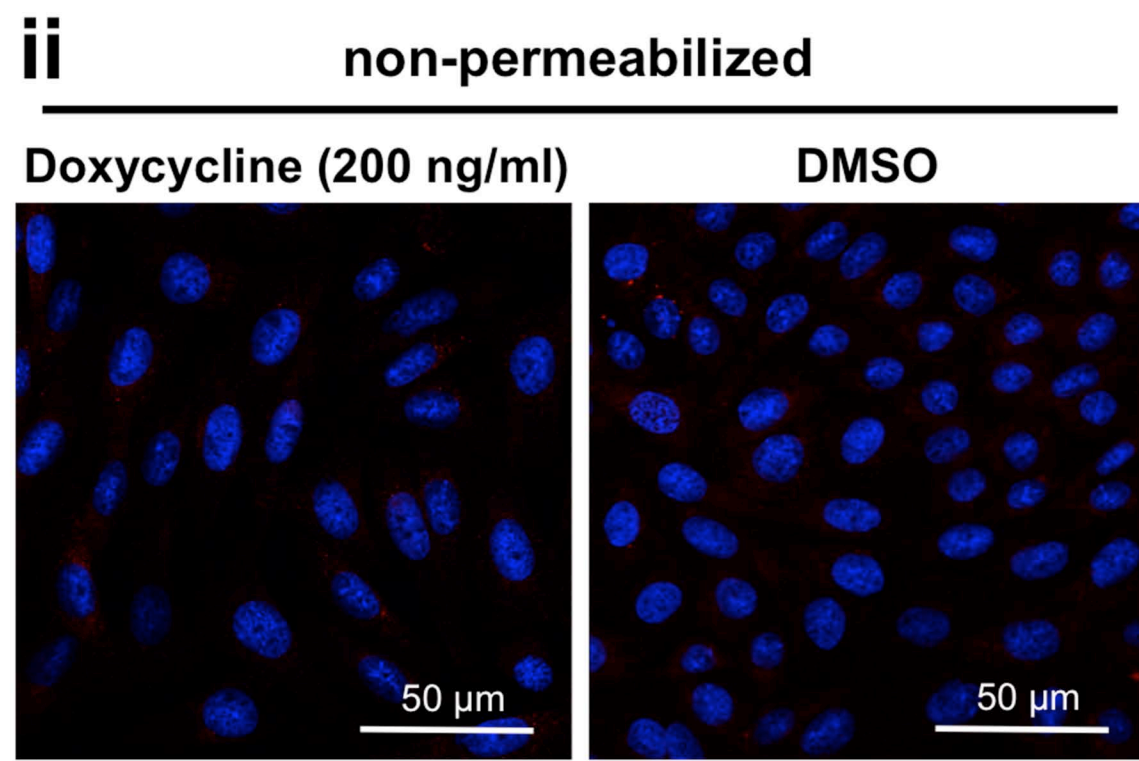
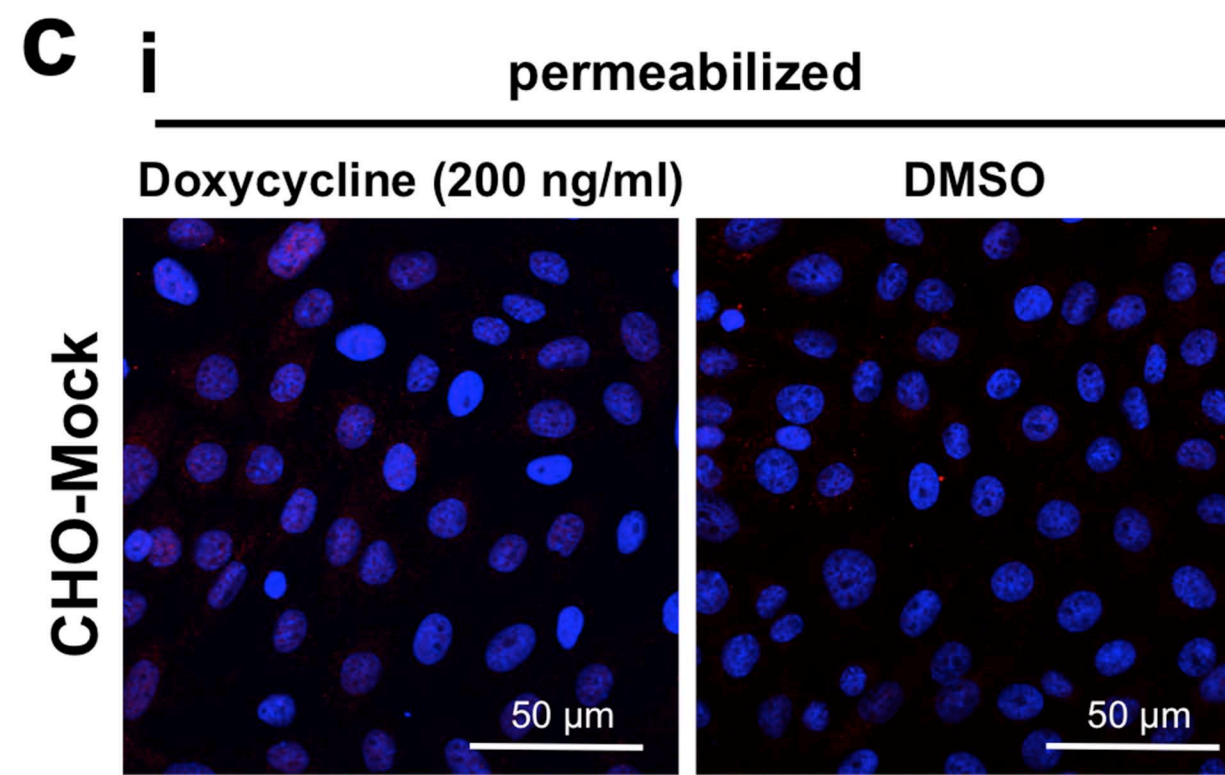
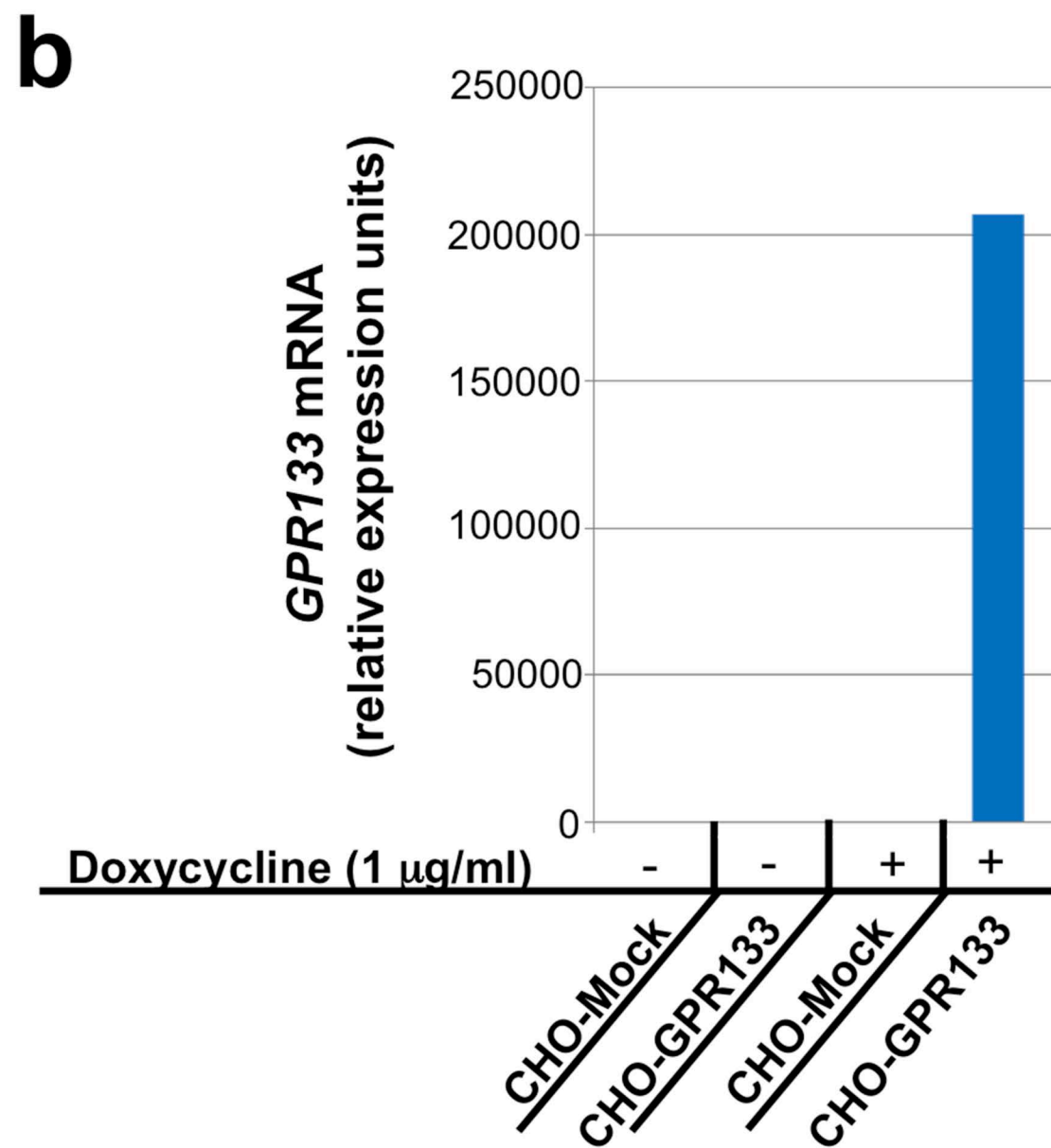
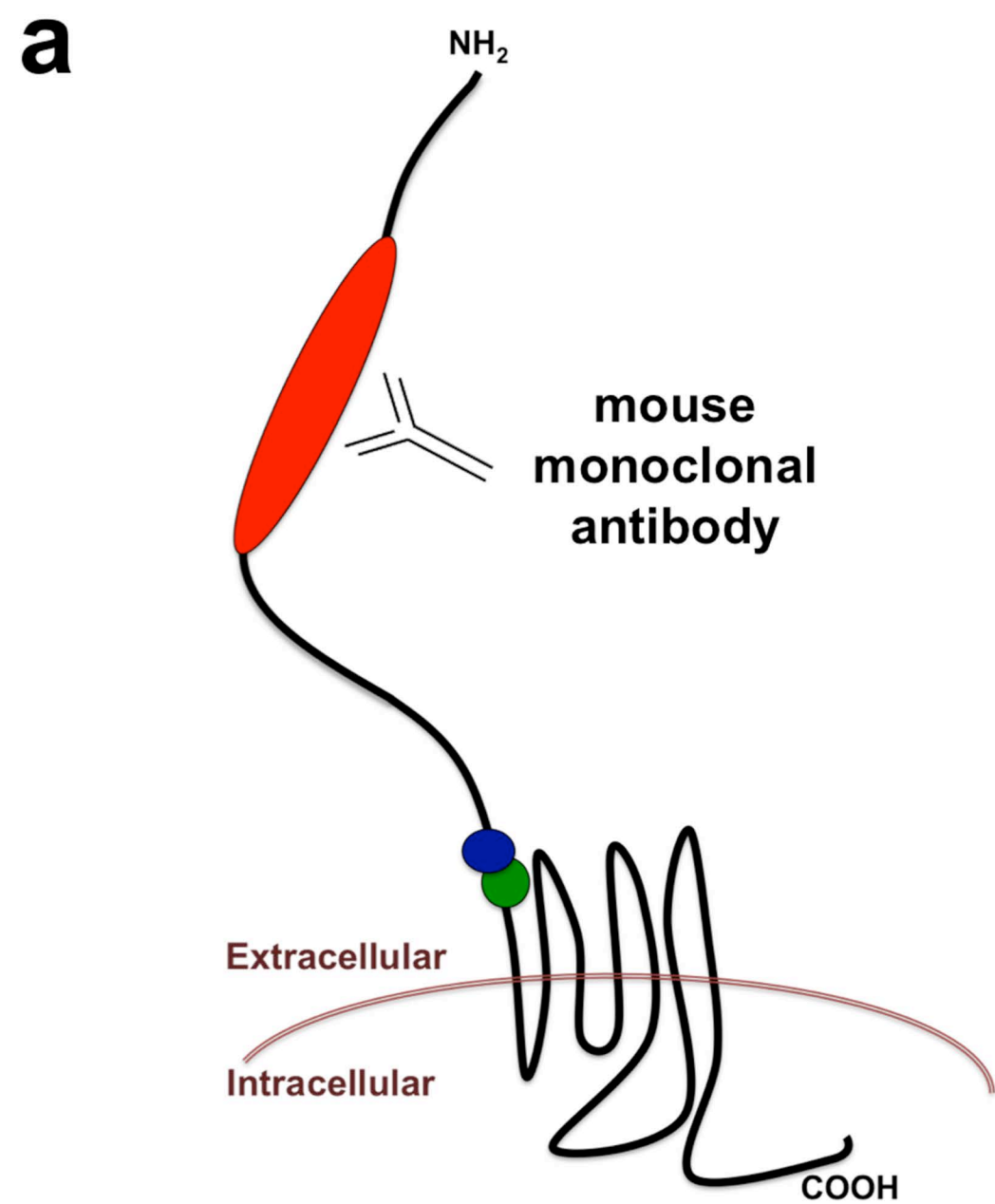


GPR133 (mouse Ab)/CA9/DAPI



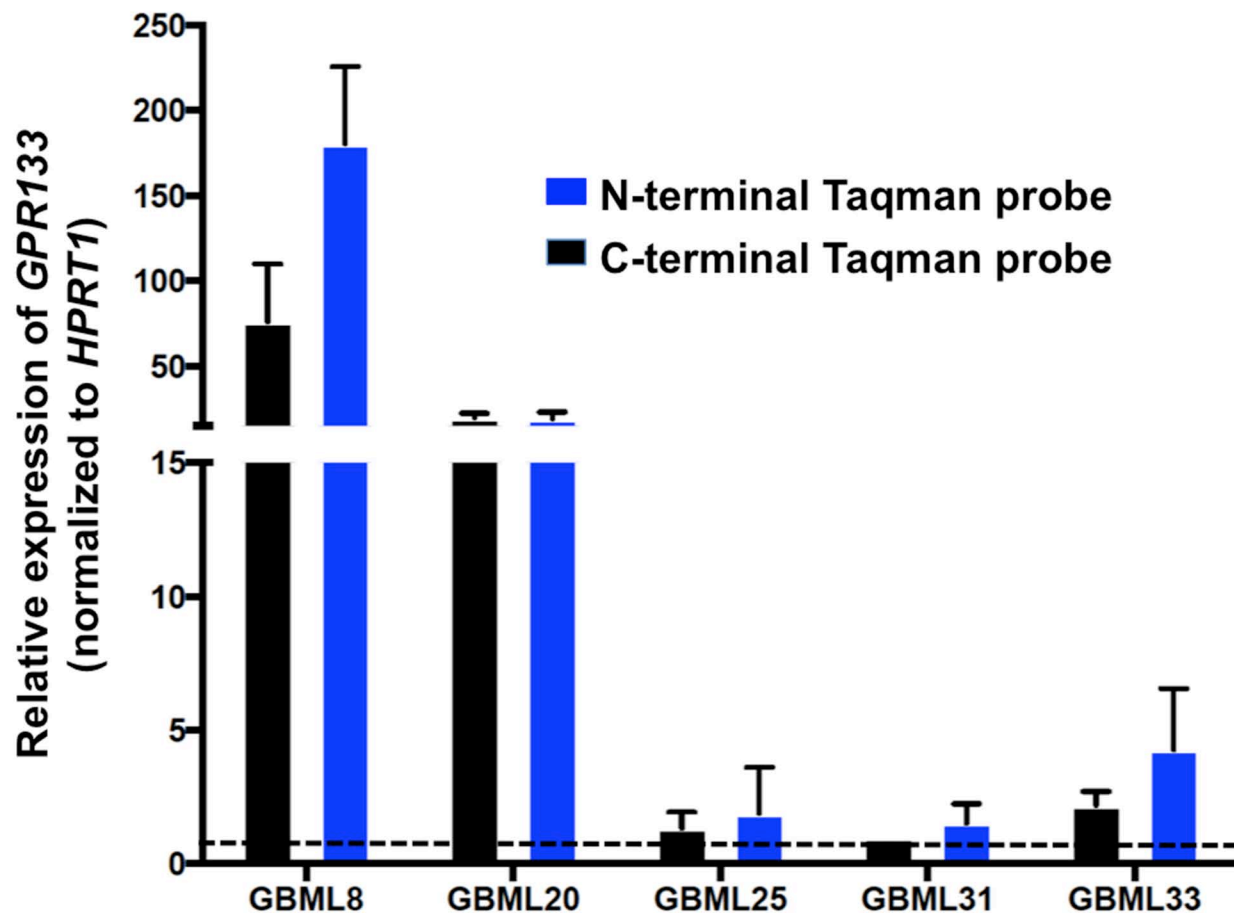
b pimonidazole/GPR133 (rabbit Ab)/EvansBlue/DAPI





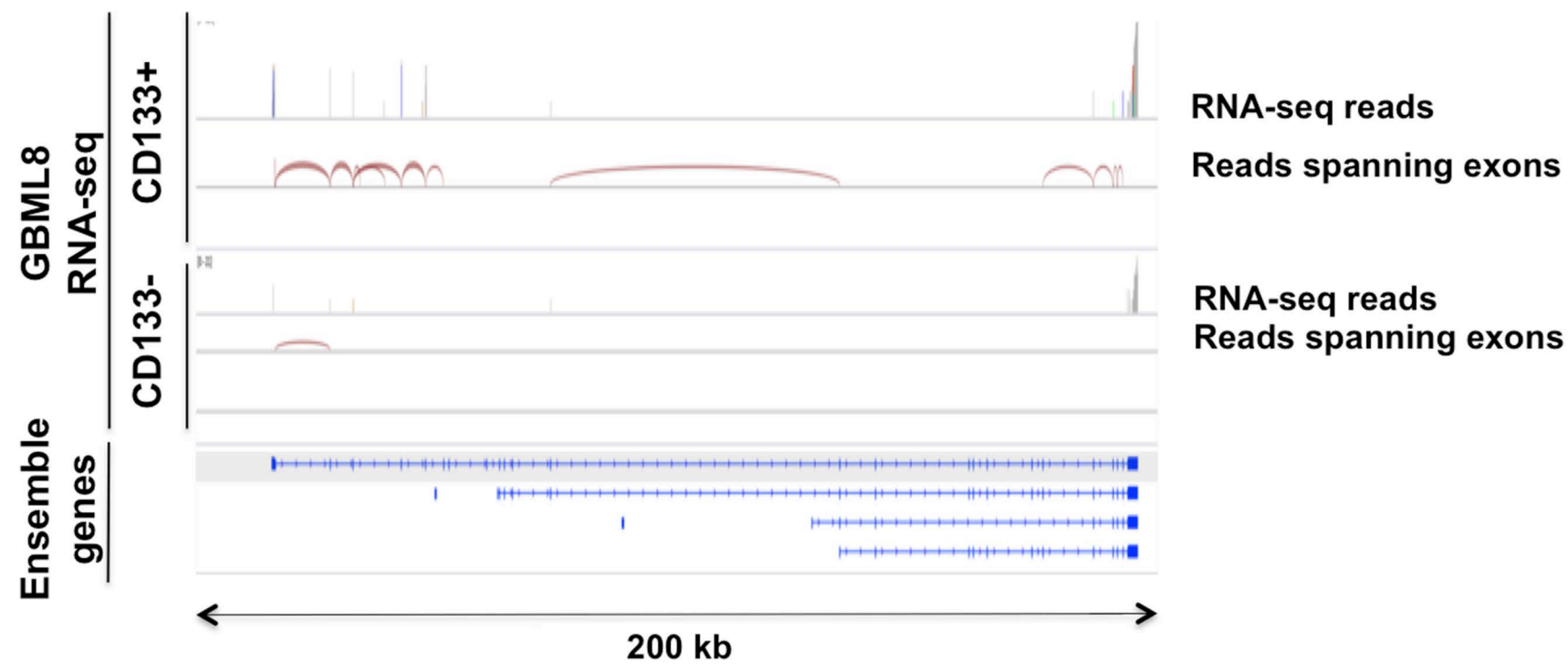
Supplementary Figure 5

a

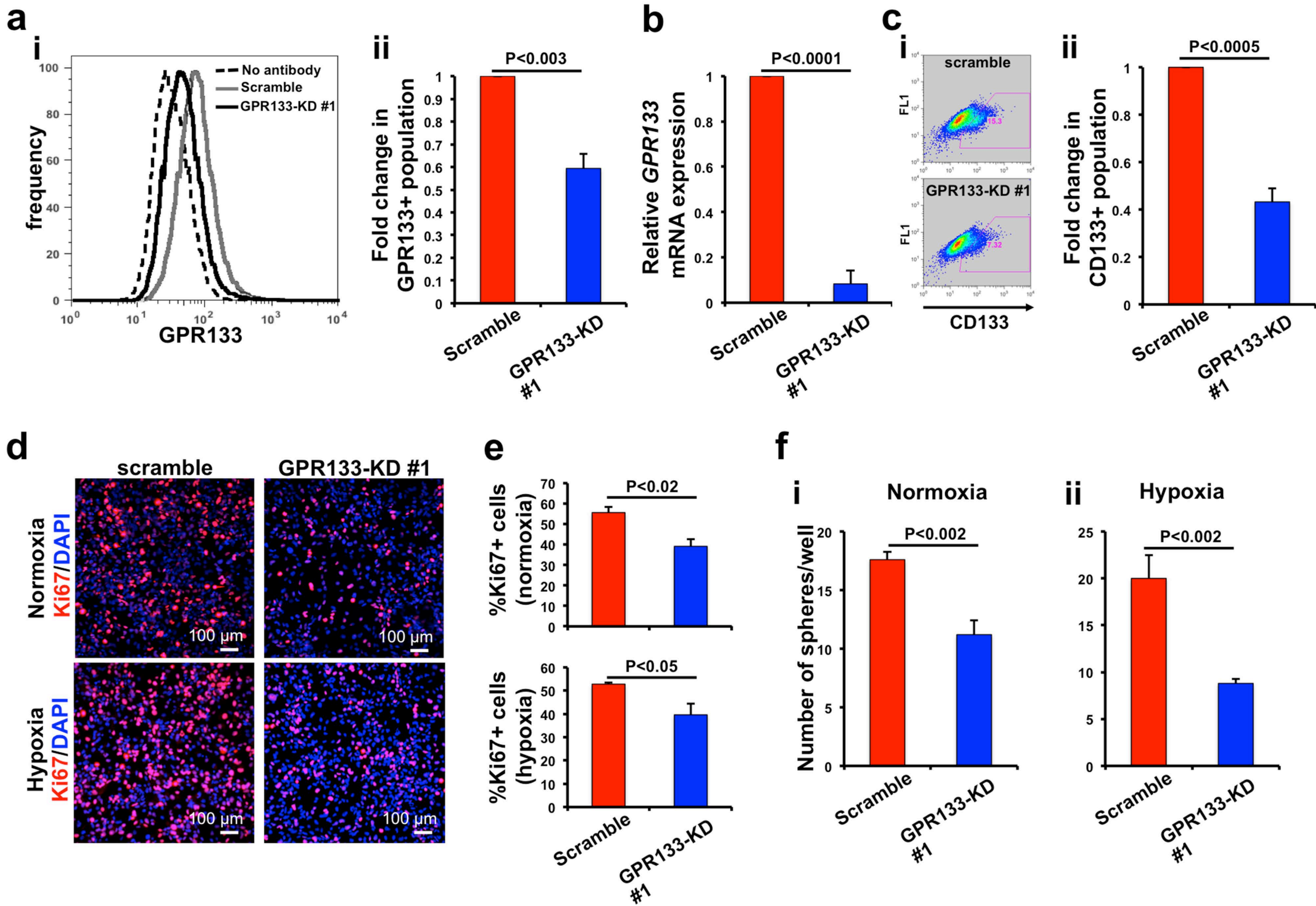


b

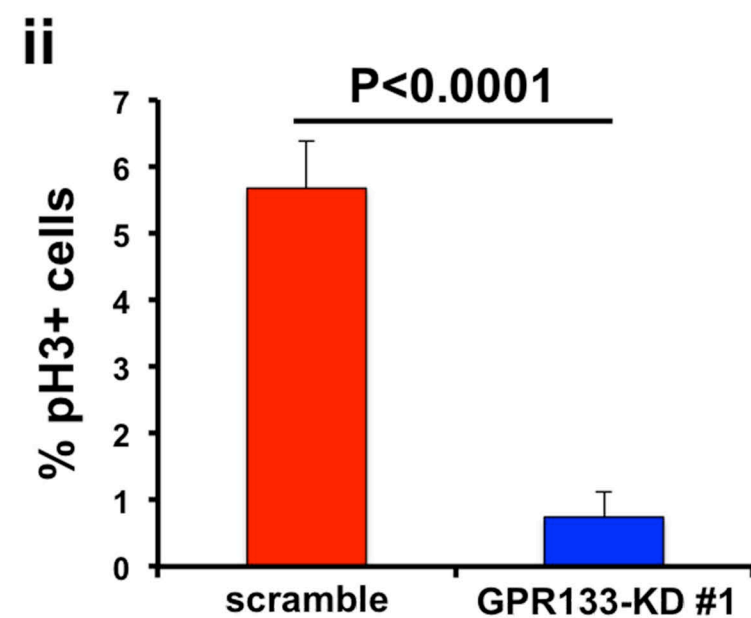
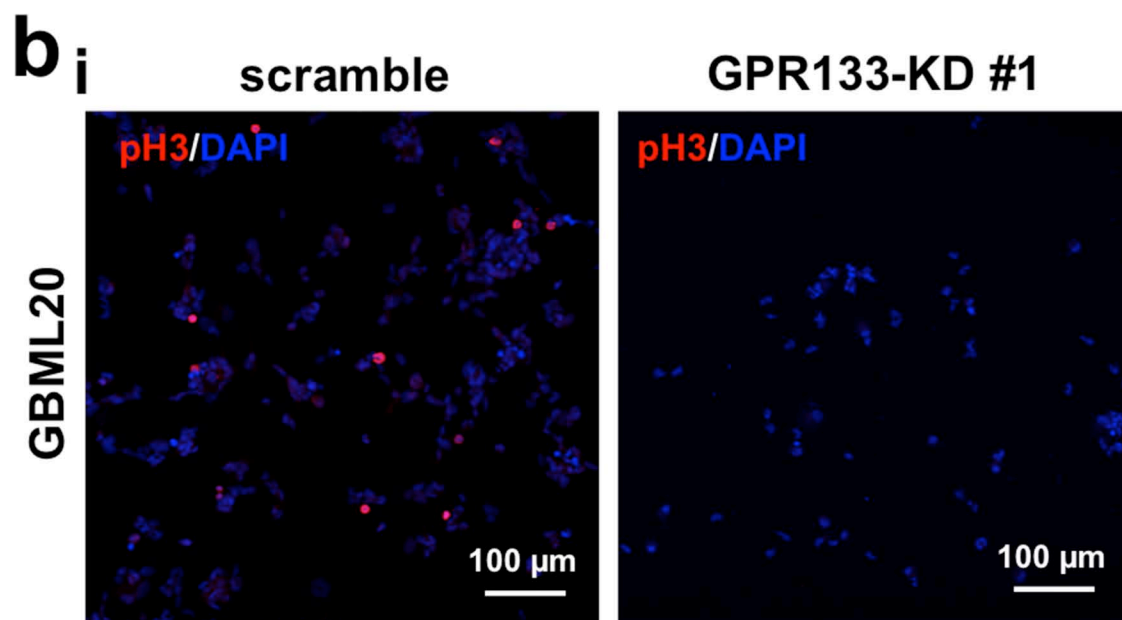
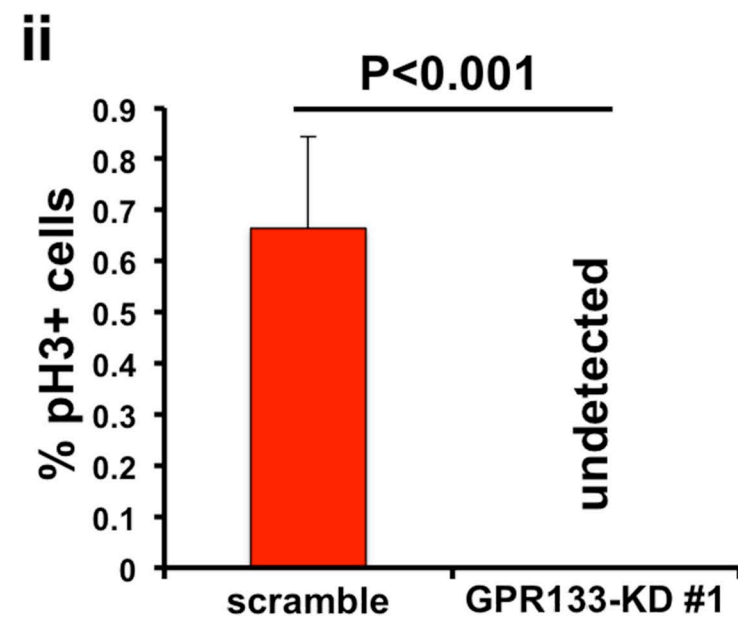
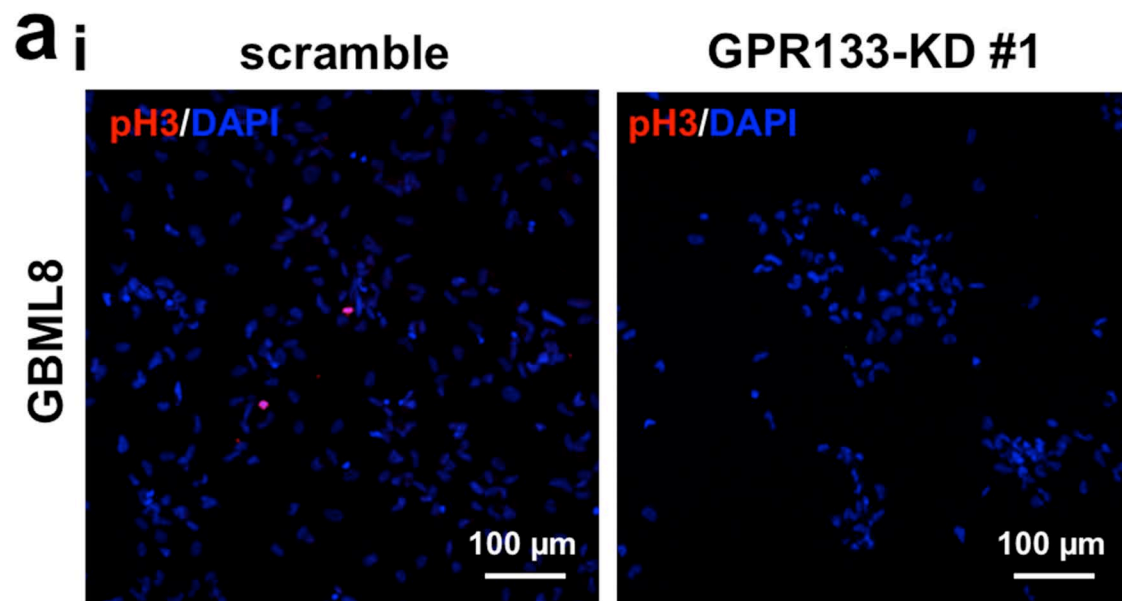
GPR133 locus



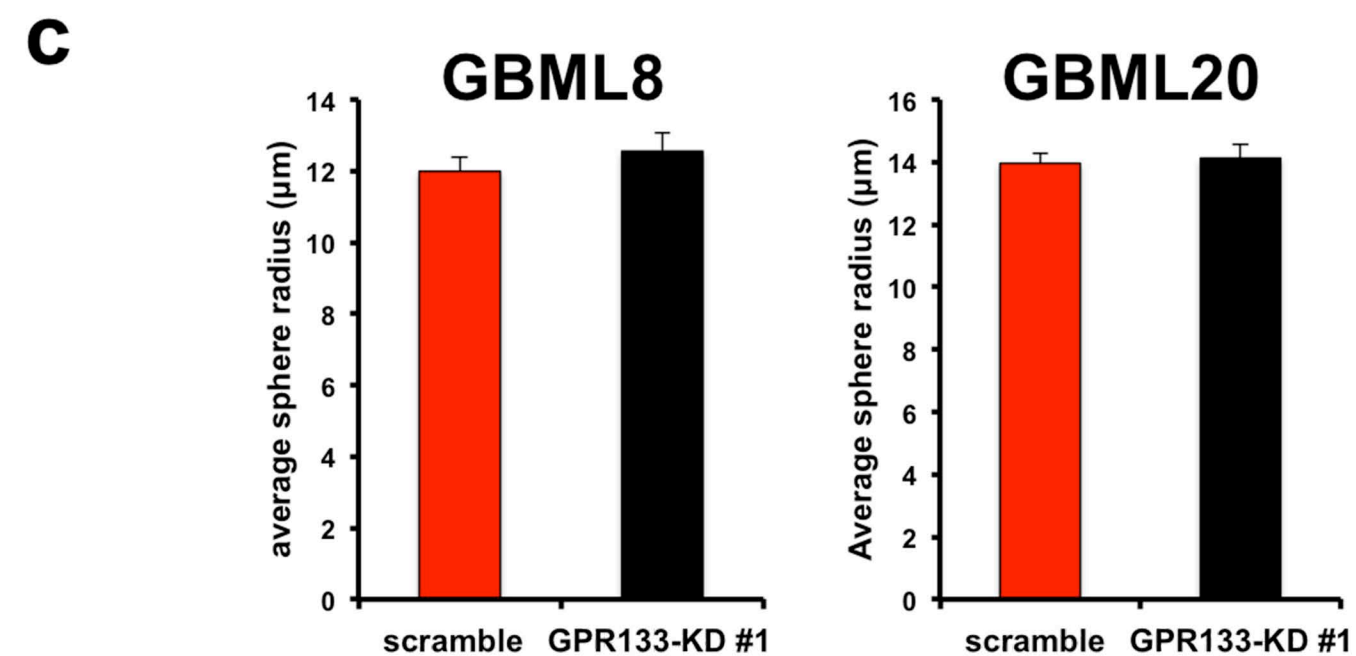
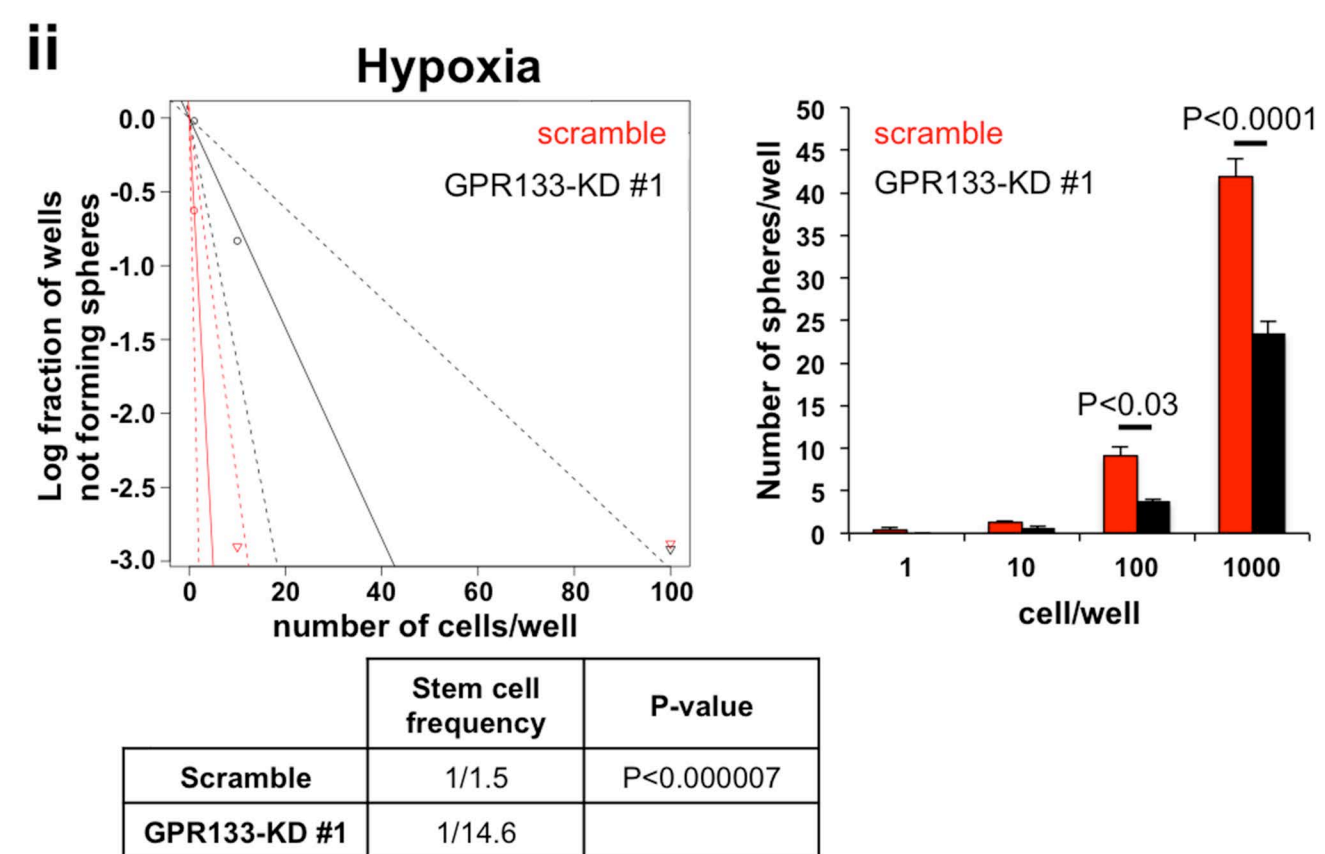
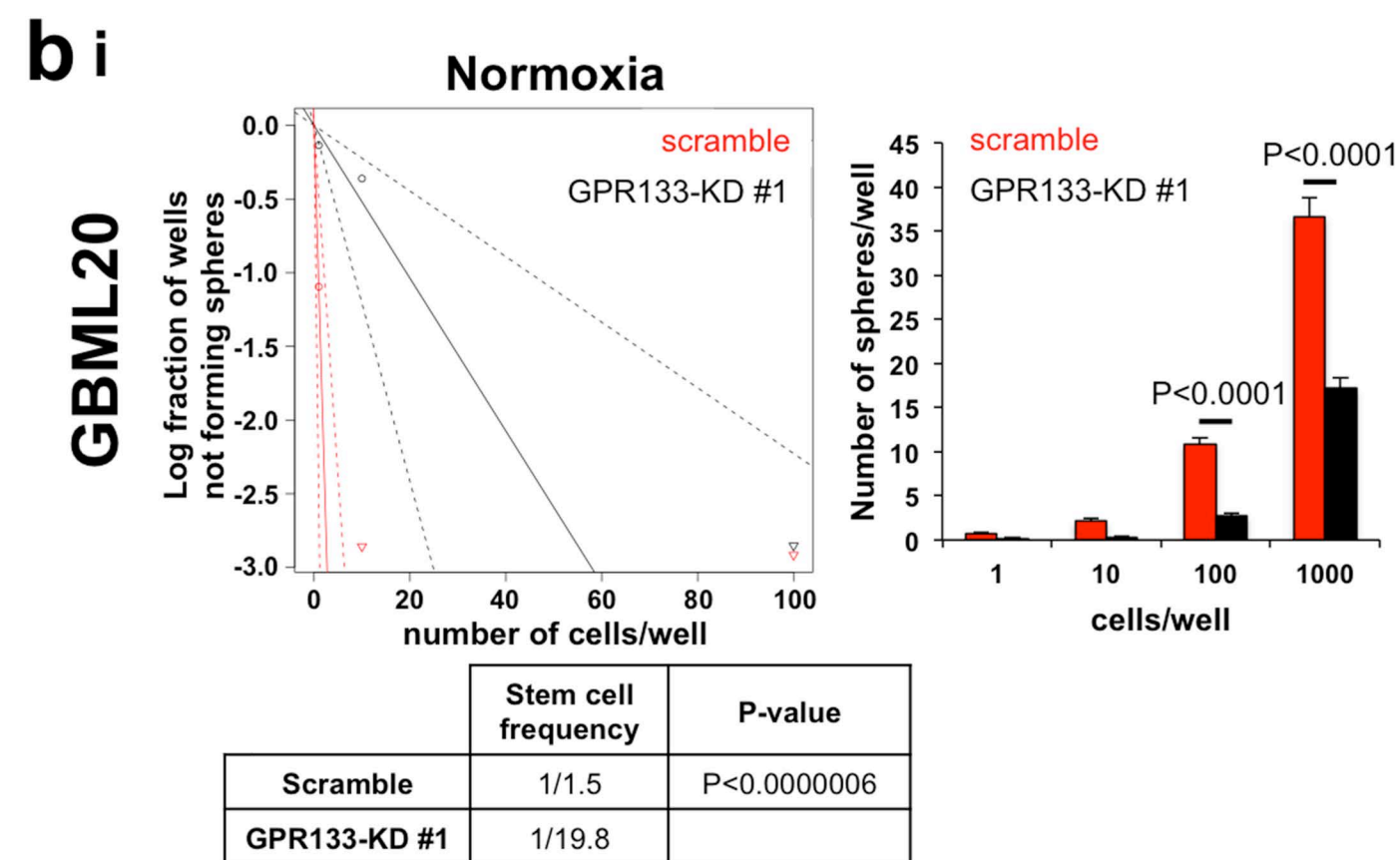
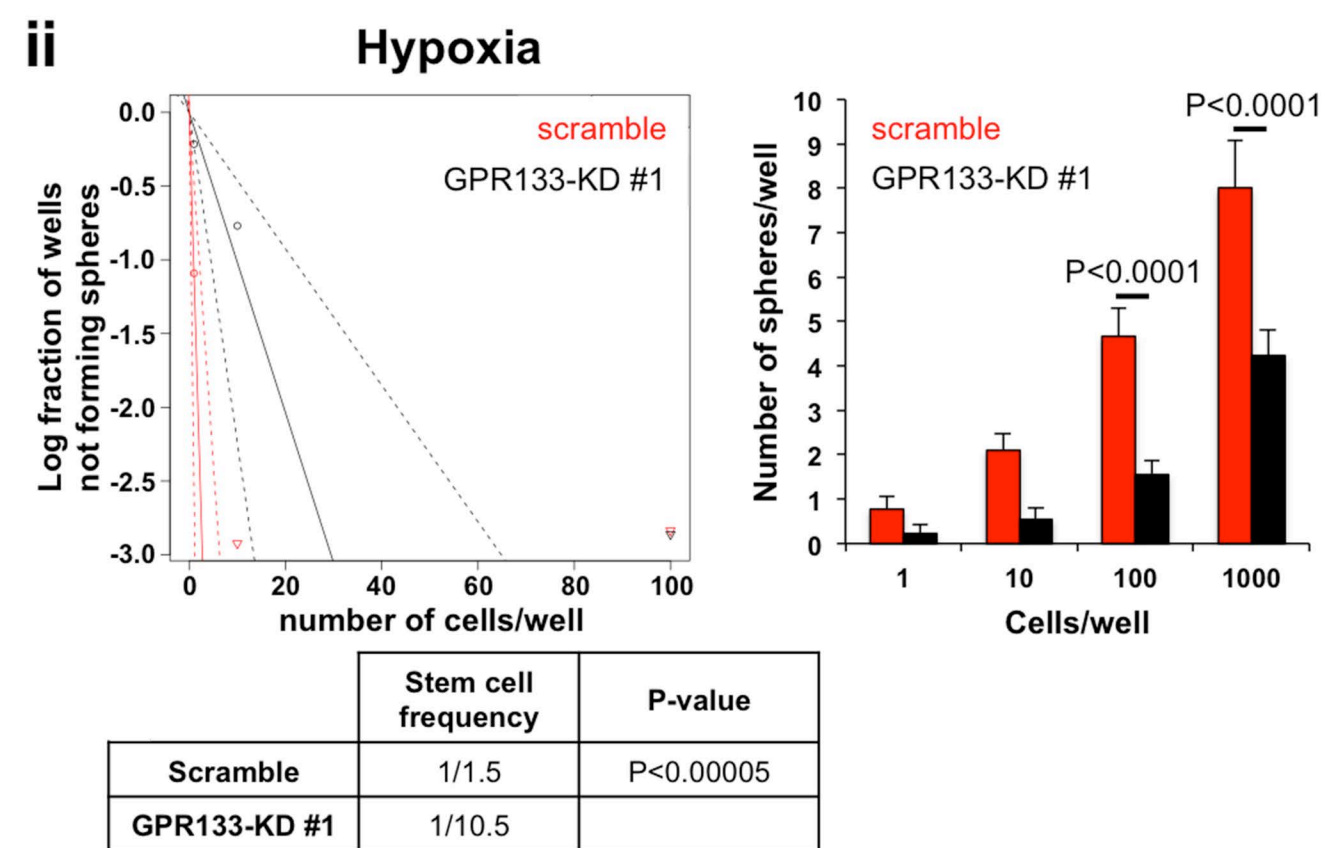
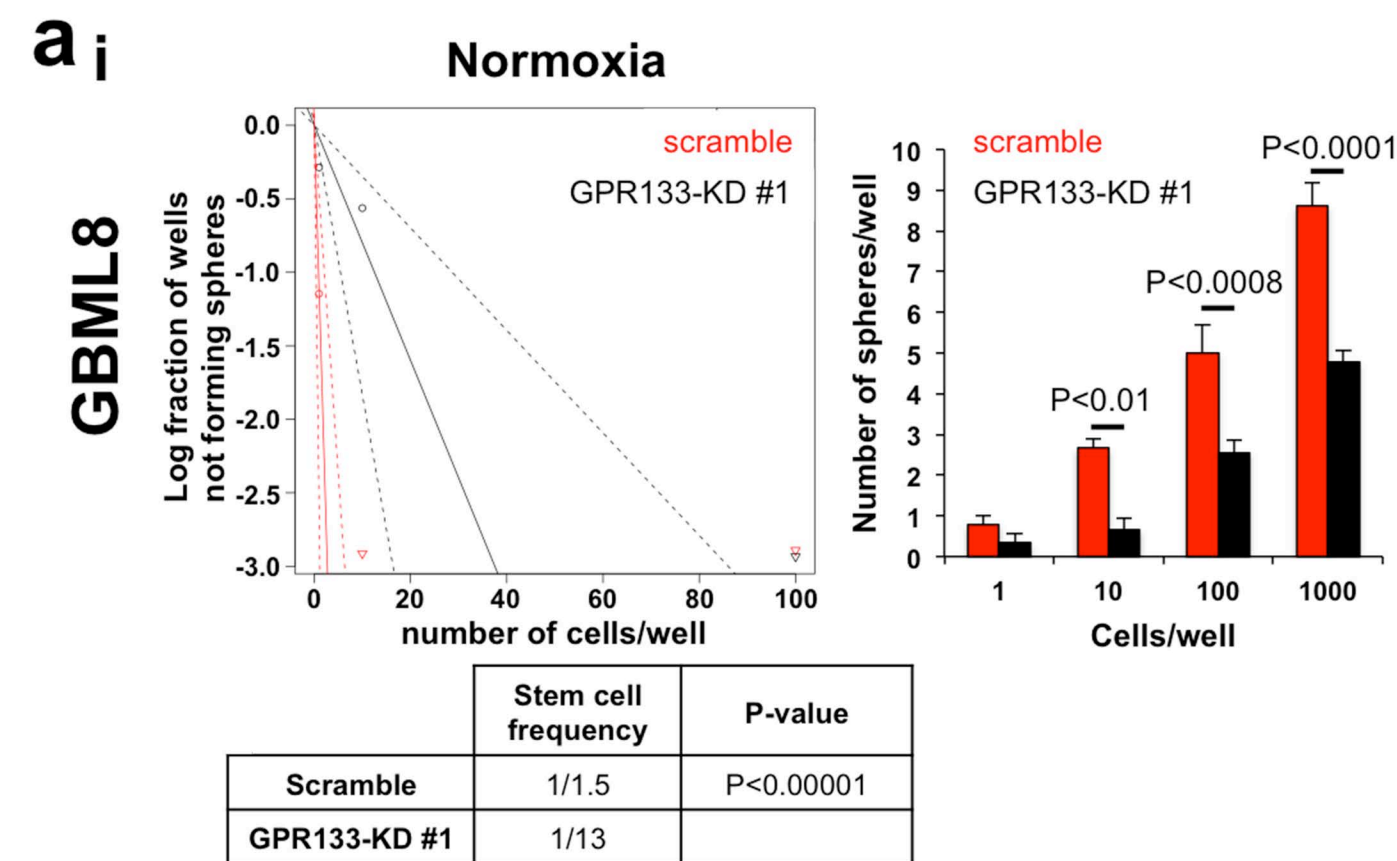
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Table 1a: Genes upregulated in CD133+ vs. CD133- GBM cells

gene	log2 change	fold change	FDR-adjusted p value
<i>PROM1</i>	4.02359	16.26377211	0.004
<i>ACAN</i>	3.77966	13.73380995	0.004
<i>IGSF21</i>	3.6893	12.90000751	0.004
<i>ALPK2</i>	3.50818	11.37803879	0.025
<i>FAM180A</i>	3.4572	10.98299791	0.004
<i>SORCS3</i>	3.37402	10.36767145	0.004
<i>ANO1</i>	3.17698	9.044119158	0.004
<i>NPTX1</i>	3.15352	8.898239933	0.004
<i>ESPN</i>	3.15049	8.879571141	0.016
<i>CA9</i>	3.07609	8.433257464	0.004
<i>GPR133</i>	3.0061	8.033897194	0.004
<i>FAM150B</i>	2.98885	7.938409582	0.004
<i>SPNS2</i>	2.94832	7.718497304	0.004
<i>DHRS3</i>	2.92511	7.595316059	0.047
<i>SLC6A6</i>	2.89976	7.463022316	0.004
<i>NRXN3</i>	2.82332	7.077893236	0.004
<i>EDN2</i>	2.67708	6.39560128	0.007
<i>ADCY8</i>	2.56274	5.908287377	0.004
<i>RHBDF2</i>	2.55968	5.895769001	0.004
<i>PCDH8</i>	2.46046	5.503921906	0.004
<i>SHANK2</i>	2.45849	5.49641143	0.004
<i>GRIP2</i>	2.30228	4.932366489	0.004
<i>CHI3L1</i>	2.25978	4.789184449	0.045
<i>GMPR</i>	2.24373	4.736199986	0.004
<i>HAPLN3</i>	2.21446	4.641078179	0.004
<i>SORCS2</i>	2.17459	4.514574438	0.004
<i>LOC154761</i>	2.11182	4.322362289	0.004
<i>TPPP3</i>	2.10823	4.3116199	0.004
<i>SPAG4</i>	2.10036	4.288163755	0.007
<i>COL15A1</i>	2.09563	4.274127662	0.004
<i>LOXL2</i>	2.08287	4.236491575	0.004
<i>SLC14A1</i>	2.08245	4.235258419	0.004
<i>CAV1</i>	2.05299	4.149650983	0.004
<i>CDH5</i>	2.05091	4.14367255	0.004
<i>PRSS35</i>	2.04238	4.119245183	0.036
<i>MMP17</i>	2.03999	4.112426801	0.004
<i>HIC1</i>	2.0392	4.110175509	0.023
<i>MAP7D2</i>	2.02911	4.081529826	0.004

<i>LMO1</i>	2.0216	4.060338483	0.004
<i>SMAD7</i>	2.00634	4.017616893	0.004
<i>ESRRB</i>	1.999	3.997228372	0.016
<i>MT1E</i>	1.99553	3.987625708	0.018
<i>C1QTNF5</i>	1.99517	3.986630788	0.007
<i>OLFM1</i>	1.98693	3.963925918	0.004
<i>TNFAIP2</i>	1.98364	3.954896665	0.048
<i>EDN1</i>	1.98353	3.954595131	0.011
<i>LOC284578</i>	1.98208	3.950622509	0.007
<i>GABRG3</i>	1.98182	3.949910599	0.018
<i>NELL2</i>	1.97029	3.918468773	0.004
<i>FAM50B</i>	1.95733	3.883426067	0.034
<i>OLFML2A</i>	1.9427	3.844244242	0.004
<i>AQP1</i>	1.93211	3.816129156	0.023
<i>H1FO</i>	1.92254	3.790898948	0.004
<i>NDRG1</i>	1.91194	3.763147922	0.004
<i>APLN</i>	1.8974	3.72541204	0.004
<i>ELFN1</i>	1.89274	3.713398127	0.013
<i>WNT7A</i>	1.86552	3.643992526	0.004
<i>STC1</i>	1.84502	3.592579295	0.004
<i>NXPH4</i>	1.84486	3.592180887	0.004
<i>CDH7</i>	1.84446	3.591185061	0.018
<i>MYO1B</i>	1.83421	3.565760981	0.004
<i>OGDHL</i>	1.81885	3.527998628	0.004
<i>MAP3K5</i>	1.80557	3.49567243	0.004
<i>SNCB</i>	1.78044	3.435309303	0.004
<i>DUSP26</i>	1.7681	3.406050912	0.039
<i>SERPINF1</i>	1.75522	3.375777926	0.007
<i>OPCML</i>	1.74628	3.354923802	0.004
<i>PCSK1</i>	1.7144	3.281601372	0.004
<i>KCNK3</i>	1.71425	3.281260195	0.018
<i>SELM</i>	1.71122	3.274376006	0.025
<i>DDIT4L</i>	1.69559	3.239093245	0.004
<i>AMPD3</i>	1.67555	3.194411122	0.004
<i>INHBB</i>	1.67535	3.193968313	0.004
<i>CEBPD</i>	1.67203	3.186626648	0.004
<i>CDK18</i>	1.66736	3.176328224	0.004
<i>ADM</i>	1.65296	3.144781975	0.004
<i>WNT7B</i>	1.64147	3.119835582	0.004
<i>CYB5R2</i>	1.63011	3.095365988	0.023
<i>GALNTL4</i>	1.625	3.084421651	0.004

<i>ADAMTS7</i>	1.6153	3.063753017	0.013
<i>LGALS3</i>	1.61346	3.059848026	0.004
<i>SIK1</i>	1.60679	3.045734107	0.004
<i>FOSL2</i>	1.60012	3.031685291	0.004
<i>CAPG</i>	1.5931	3.016969266	0.004
<i>SGIP1</i>	1.58736	3.004989604	0.004
<i>NRP1</i>	1.57982	2.989325506	0.004
<i>ACCS</i>	1.57952	2.988703958	0.021
<i>MRVI1</i>	1.5783	2.98617766	0.004
<i>KBTBD11</i>	1.57501	2.979375578	0.004
<i>SLC16A3</i>	1.573	2.975227525	0.004
<i>CRABP2</i>	1.5722	2.973578166	0.004
<i>HSF4</i>	1.5596	2.947721039	0.004
<i>AKAP12</i>	1.55173	2.931684799	0.004
<i>COL23A1</i>	1.54094	2.909840348	0.032
<i>HSD11B2</i>	1.54088	2.909719334	0.039
<i>CNGA3</i>	1.53308	2.894030246	0.013
<i>BAMBI</i>	1.52091	2.869720041	0.039
<i>ALDOC</i>	1.51809	2.86411615	0.004
<i>VWA1</i>	1.47682	2.783345494	0.011
<i>EGLN3</i>	1.47663	2.782978957	0.004
<i>CHST15</i>	1.47479	2.779431834	0.021
<i>MFSD4</i>	1.47137	2.772850818	0.007
<i>PYGL</i>	1.47036	2.770910283	0.004
<i>PDZD2</i>	1.46249	2.755835932	0.004
<i>IL17RC</i>	1.46152	2.753983661	0.045
<i>ITPR1</i>	1.44994	2.731966892	0.004
<i>CLIC6</i>	1.43228	2.69872879	0.038
<i>RNF182</i>	1.42411	2.683489048	0.004
<i>GSN</i>	1.41736	2.670963017	0.004
<i>EFR3B</i>	1.41381	2.664398731	0.004
<i>ADSSL1</i>	1.40184	2.642383745	0.004
<i>INSIG2</i>	1.39879	2.636803386	0.004
<i>S1PR3</i>	1.39323	2.62666097	0.004
<i>TCEAL5</i>	1.39179	2.624040524	0.007
<i>CA12</i>	1.38766	2.61653943	0.004
<i>EPB49</i>	1.36479	2.575388356	0.034
<i>MLLT4-AS1</i>	1.36347	2.573033071	0.021
<i>FAM69C</i>	1.36208	2.570555213	0.021
<i>TF</i>	1.34861	2.546666424	0.030
<i>BHLHE40</i>	1.34337	2.537433477	0.004

<i>PPFIA4</i>	1.34201	2.535042616	0.004
<i>FAM110C</i>	1.3388	2.52940841	0.047
<i>BEST3</i>	1.33022	2.514410148	0.004
<i>MIAT</i>	1.31799	2.493185107	0.004
<i>NT5E</i>	1.3026	2.466730322	0.004
<i>FAM115C</i>	1.29649	2.456305491	0.004
<i>FGF11</i>	1.2949	2.453599878	0.038
<i>OBSL1</i>	1.29456	2.453021706	0.004
<i>ELL2</i>	1.28918	2.4438911	0.004
<i>TMEM123</i>	1.2846	2.436144991	0.004
<i>ZNF395</i>	1.28442	2.435841061	0.004
<i>PCOLCE2</i>	1.28285	2.43319172	0.007
<i>ID2</i>	1.27942	2.427413691	0.004
<i>SLC2A1</i>	1.2714	2.413957038	0.004
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<i>LOC100216479</i>	1.26444	2.40233942	0.004
<i>GPR12</i>	1.26042	2.395654735	0.004
<i>PFKFB4</i>	1.24961	2.377771366	0.004
<i>HSPB6</i>	1.24708	2.373605212	0.004
<i>RCOR2</i>	1.2457	2.371335842	0.004
<i>MAF</i>	1.24142	2.364311296	0.004
<i>GATM</i>	1.23682	2.35678475	0.004
<i>ZNF334</i>	1.23033	2.346206505	0.023
<i>STC2</i>	1.21213	2.316794367	0.004
<i>NTRK2</i>	1.20587	2.30676334	0.004
<i>SLC43A3</i>	1.20298	2.302147068	0.004
<i>EHD2</i>	1.19712	2.292815075	0.004
<i>SH2D4A</i>	1.19615	2.291274012	0.016
<i>IGFBP5</i>	1.19567	2.290511808	0.004
<i>GYPC</i>	1.19441	2.288512227	0.007
<i>FAM49A</i>	1.19233	2.285215151	0.004
<i>MGST1</i>	1.18968	2.281021429	0.045
<i>HILPDA</i>	1.18768	2.277861453	0.004
<i>PTPRD</i>	1.17923	2.2645588	0.004
<i>SEMA5B</i>	1.17591	2.259353479	0.016
<i>SLC2A3</i>	1.16547	2.24306278	0.004
<i>LMO2</i>	1.16336	2.239784608	0.018
<i>NRN1</i>	1.14803	2.216110778	0.004
<i>YWHAH</i>	1.13928	2.202710659	0.004
<i>EMP2</i>	1.13889	2.202115287	0.004
<i>GABBR2</i>	1.13096	2.19004422	0.004

<i>ARHGEF3</i>	1.12941	2.187692548	0.004
<i>CNR1</i>	1.12917	2.187328644	0.004
<i>SFXN3</i>	1.12198	2.176454711	0.007
<i>KIAA1671</i>	1.11606	2.167542077	0.004
<i>TSC22D3</i>	1.11344	2.163609294	0.004
<i>MAFB</i>	1.11026	2.158845501	0.034
<i>EFS</i>	1.10827	2.155869723	0.004
<i>CD9</i>	1.10732	2.154450572	0.004
<i>PSTPIP2</i>	1.09879	2.141749869	0.045
<i>LOC254559</i>	1.09859	2.14145298	0.030
<i>PPP1R3C</i>	1.0874	2.124907441	0.004
<i>ST3GAL1</i>	1.08427	2.120302344	0.004
<i>PRPH</i>	1.08074	2.115120709	0.043
<i>FABP5</i>	1.07707	2.109746997	0.004
<i>PLOD2</i>	1.06946	2.098647697	0.004
<i>RARA</i>	1.05355	2.075631017	0.030
<i>TRIM2</i>	1.04904	2.069152531	0.004
<i>MED15</i>	1.04897	2.069052138	0.004
<i>CD97</i>	1.04399	2.061922345	0.004
<i>FKBP9L</i>	1.03953	2.055557886	0.036
<i>HK2</i>	1.03389	2.047537678	0.004
<i>PDK1</i>	1.03177	2.044531088	0.004
<i>NFATC4</i>	1.02656	2.037160995	0.013
<i>TET1</i>	1.02306	2.032224802	0.011
<i>SPEG</i>	1.01486	2.020706793	0.028
<i>MEST</i>	1.00816	2.011344214	0.004
<i>PPAP2B</i>	1.00257	2.003565952	0.004
<i>MT1X</i>	1.00088	2.001220311	0.038
<i>EPAS1</i>	0.995335	1.993543381	0.004
<i>ENO2</i>	0.993995	1.991692603	0.004
<i>TMEM45A</i>	0.991327	1.988012737	0.007
<i>B3GALNT1</i>	0.98677	1.981743164	0.004
<i>CHPF</i>	0.983777	1.977636122	0.004
<i>LINGO1</i>	0.983445	1.977181071	0.004
<i>CSPG4</i>	0.98313	1.976749418	0.011
<i>GRIK1</i>	0.978111	1.969884442	0.004
<i>TGFA</i>	0.976867	1.968186591	0.004
<i>SIPA1L2</i>	0.975836	1.966780559	0.004
<i>FLNB</i>	0.968271	1.956494426	0.018
<i>CD109</i>	0.964911	1.951943102	0.023
<i>LRRC49</i>	0.961732	1.947646702	0.007

<i>MLLT3</i>	0.955775	1.939621297	0.034
<i>VEGFA</i>	0.955466	1.939205909	0.004
<i>MXI1</i>	0.938912	1.917081937	0.004
<i>MIR210HG</i>	0.935642	1.912741617	0.039
<i>ZFP36L2</i>	0.932293	1.908306624	0.004
<i>SYTL2</i>	0.930037	1.90532486	0.004
<i>LGR6</i>	0.926289	1.900381411	0.034
<i>TMEM198</i>	0.925968	1.899958623	0.043
<i>MDK</i>	0.9257	1.899605713	0.004
<i>APOLD1</i>	0.918636	1.890327233	0.004
<i>FLJ46906</i>	0.9126	1.882434935	0.007
<i>RND3</i>	0.912391	1.882162251	0.045
<i>KCNE4</i>	0.911253	1.880678184	0.004
<i>SORL1</i>	0.904571	1.871987758	0.004
<i>SCARB1</i>	0.902987	1.869933546	0.011
<i>NPNT</i>	0.902121	1.868811426	0.013
<i>FKBP9</i>	0.899069	1.864862162	0.004
<i>NT5C3</i>	0.896159	1.861104417	0.004
<i>PDE4D</i>	0.894443	1.85889206	0.032
<i>C1orf198</i>	0.885987	1.848028492	0.004
<i>PPARGC1B</i>	0.885446	1.847335625	0.004
<i>FGFR1</i>	0.869414	1.826920683	0.004
<i>CABIN1</i>	0.863656	1.819643716	0.013
<i>ERO1L</i>	0.858669	1.813364567	0.018
<i>MEG3</i>	0.858232	1.812815372	0.023
<i>IFITM3</i>	0.857451	1.811834274	0.028
<i>LOC730101</i>	0.855987	1.809996616	0.043
<i>FRAS1</i>	0.848884	1.801107136	0.004
<i>AMD1</i>	0.846678	1.798355199	0.004
<i>TMEM22</i>	0.843261	1.794100863	0.025
<i>RORA</i>	0.843026	1.793808647	0.013
<i>MT3</i>	0.842154	1.792724753	0.004
<i>SBF2</i>	0.841085	1.791396882	0.004
<i>ARL4C</i>	0.84046	1.790620986	0.004
<i>IQSEC1</i>	0.839238	1.789104926	0.032
<i>HJURP</i>	0.836282	1.785442905	0.013
<i>C11orf41</i>	0.829182	1.77667771	0.023
<i>EYA2</i>	0.828153	1.77541095	0.025
<i>NOTCH3</i>	0.826995	1.773986462	0.013
<i>KATNAL1</i>	0.826141	1.772936666	0.018
<i>CSGALNACT1</i>	0.819128	1.764339261	0.011

<i>PSMD5</i>	0.814537	1.758733636	0.021
<i>S1PR2</i>	0.814434	1.758608077	0.050
<i>JAM2</i>	0.8112	1.75467033	0.032
<i>RP9P</i>	0.80781	1.7505521	0.030
<i>CXCR4</i>	0.806631	1.749122097	0.025
<i>VOPP1</i>	0.796003	1.736284066	0.007
<i>NCALD</i>	0.794744	1.73476952	0.011
<i>CAV2</i>	0.78674	1.725171754	0.021
<i>C14orf43</i>	0.785832	1.724086311	0.016
<i>NEDD4L</i>	0.783422	1.721208655	0.023
<i>GRB10</i>	0.782665	1.720305752	0.007
<i>TNIP1</i>	0.773008	1.708828958	0.023
<i>TSKU</i>	0.771003	1.706455745	0.043
<i>AK4</i>	0.758256	1.691444688	0.004
<i>PPFIBP2</i>	0.754097	1.686575614	0.023
<i>LPL</i>	0.75126	1.683262292	0.016
<i>CCNG2</i>	0.74887	1.680476071	0.045
<i>FEM1C</i>	0.737755	1.667578874	0.039
<i>DDIT4</i>	0.711344	1.637328726	0.036
<i>PSPH</i>	0.702172	1.626952352	0.016
<i>C5orf62</i>	0.687785	1.610808511	0.038
<i>BNIP3L</i>	0.662611	1.582944858	0.043
<i>ARHGAP21</i>	0.660533	1.580666489	0.045

Supplementary Table 1b: Genes downregulated in CD133+ vs. CD133- GBM cells

gene	log2 change	fold change	FDR-adjusted p value
KCNJ6	2.82636	7.092823266	0.004
LRRN1	2.40211	5.285756626	0.004
ID3	2.35582	5.118850954	0.004
IL33	2.2916	4.895987925	0.011
SCRG1	1.97385	3.928149942	0.004
NEUROD1	1.86324	3.638238197	0.004
ID1	1.75405	3.37304134	0.004
ST6GAL1	1.67493	3.193038614	0.004
SPP1	1.5431	2.914200218	0.004
SNCAIP	1.52535	2.87856542	0.011
GRIK3	1.47713	2.783943631	0.004
GPR126	1.39706	2.633643372	0.039
EDNRB	1.39034	2.621404521	0.004
DCC	1.38744	2.616140458	0.016
HOPX	1.36608	2.577692195	0.013
TMEM108	1.35151	2.551790695	0.004
IQGAP2	1.29641	2.456169288	0.004
TIMP3	1.26842	2.408975966	0.032
KIAA1598	1.22756	2.341706069	0.004
BCAN	1.22132	2.331599504	0.004
PLAT	1.20322	2.302530074	0.004
CDH6	1.19191	2.284549972	0.004
POSTN	1.17074	2.251271415	0.004
VGF	1.16919	2.248853998	0.004
TNFRSF19	1.14168	2.206378035	0.004
HSPA12A	1.07027	2.099826312	0.004
SLC1A2	1.05194	2.073315973	0.007
KCNQ2	1.03411	2.047849936	0.004
CD24	1.01029	2.014315962	0.004
LAMB1	0.957362	1.941756102	0.018
FLRT3	0.944393	1.924379052	0.013
PVRL3	0.937611	1.915353922	0.004
BDNF	0.93551	1.912566618	0.041
VCAN	0.903931	1.871157501	0.048
SHROOM3	0.887019	1.849350912	0.013
LRRC17	0.885076	1.84686191	0.041
FST	0.884673	1.846346083	0.048
RCAN1	0.873007	1.831476259	0.016

ANXA1	0.871862	1.830023278	0.004
ODZ2	0.869303	1.826780127	0.016
C8orf46	0.800004	1.741105954	0.048
ALCAM	0.77189	1.707505233	0.034
WISP1	0.754421	1.686954427	0.032
EYA4	0.741246	1.671618928	0.023
SACS	0.740583	1.670850901	0.021
DLC1	0.725233	1.653167607	0.038
COL5A2	0.719459	1.646564469	0.039
SPRY4	0.708049	1.633593465	0.03

Supplementary Table 2a. Taqman Assays

Target	TaqMan Assay
<i>GPR133</i> -C-terminal	Hs00914797_m1
<i>GPR133</i> -N-terminal	Hs00138665_m1
<i>HPRT1</i>	Hs28000695_m1
<i>GAPDH</i>	Hs02758991_m1
<i>PROM1 (CD133)</i>	Hs00195682_m1
<i>HIF1A</i>	Hs00153153_m1
<i>18S</i>	Hs99999901_s1

Supplementary Table 2b. PCR primers used in the study

Target	Forward primer	Reverse primer	Amplicon (bp)
<i>GPR133</i> (in CHO cells)	5'CCAGAGACAACAGCATGACC ^{3'}	5'TTTGGACTTCCAGGTGAACA ^{3'}	86
<i>GPR133</i> promoter HRE	5'CTGTGTGTCCACGTGTATTTGG ^{3'}	5'AGAAAAGAGTCAGCCCCTTCG ^{3'}	90
<i>CA9</i> promoter HRE	5'GACTTTGGCTCCATCTCTGC ^{3'}	5'GTGTGTACGGGCTGTACGTG ^{3'}	133

Supplementary Table 2c. shRNA constructs

shRNA construct	Target sequence
<i>GPR133</i> target #1	5'CCTGCAGGGACTGTTTCATATT ^{3'}
<i>GPR133</i> target #2	5'GGAGTCACGCTTCTCTATTAC ^{3'}
<i>HIF1A</i>	5'GCCGCTCAATTTATGAATATT ^{3'}
<i>scramble</i>	5'CCTAAGGTTAAGTCGCCCTCG ^{3'}

Supplementary Table 3. Antibodies used for Immunofluorescence staining

Antigen	Company/Catalog #	Dilution
GPR133 (polyclonal)	LSBio/ LS-A2034	1:200
GPR133 (monoclonal)		1:1000 (1:5000 in CHO cell assays)
CD133 (W6B3C1)	Miltenyi/130-092-395	1:50
Ki67	Abcam/ ab15580	1:200
pH3	EMD Millipore/06-570	1:1000
Hif1α	Bethyl Labs/ A300-286A	1:200
CA9	Abcam/ ab15086	1:200
Hydroxy-probe (pimonidazole)	HydroxyProbe/ HP1-1000	1:100
hNA	Millipore/ MAB1281	1:200