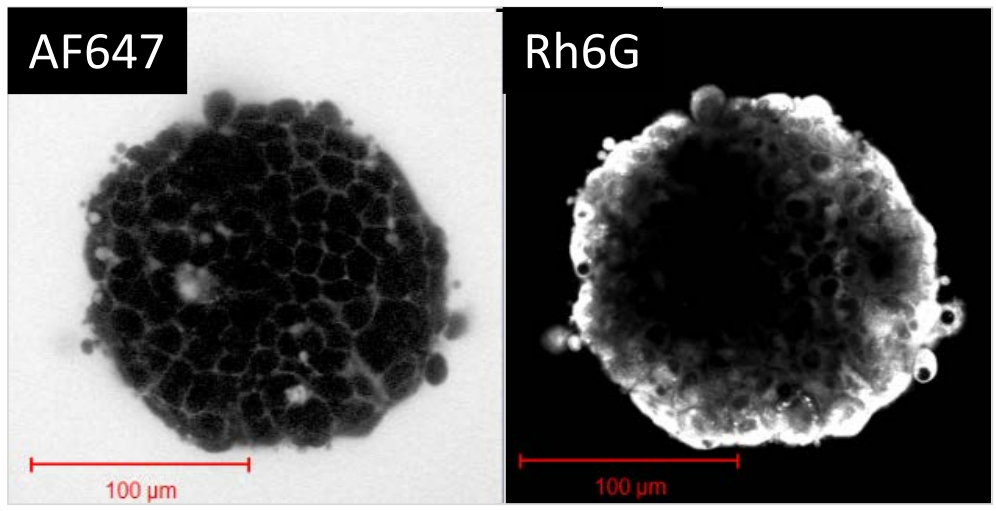
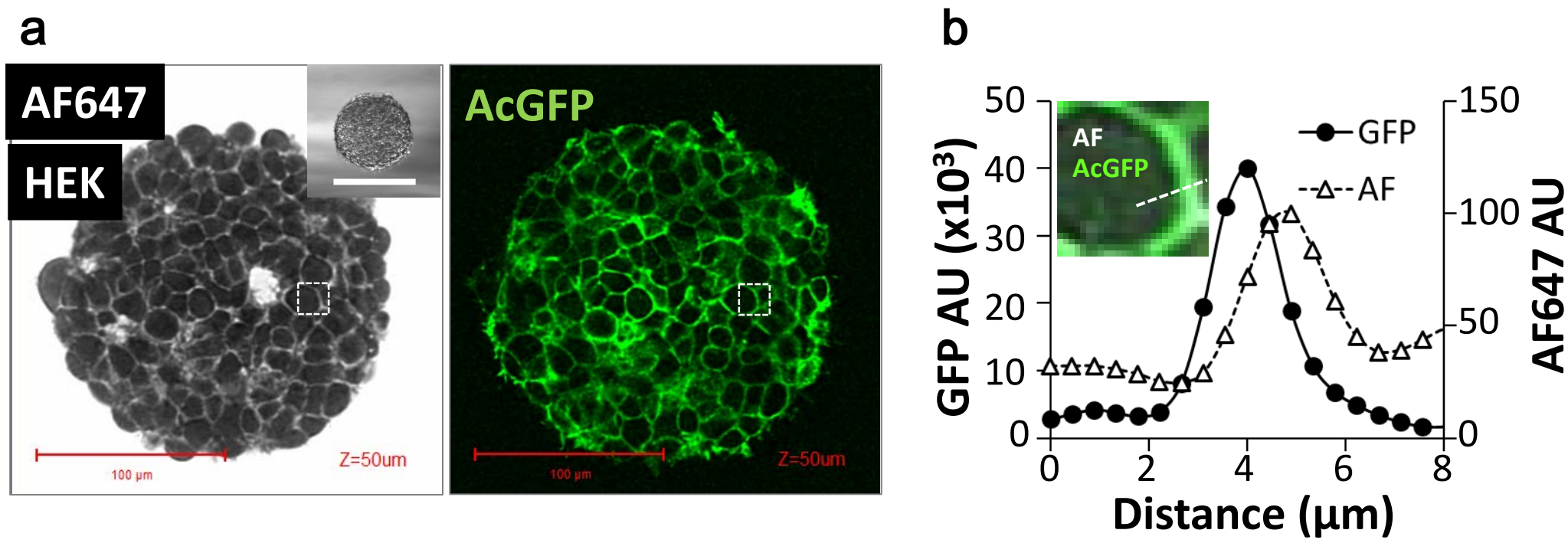
Extended Data Figure 1



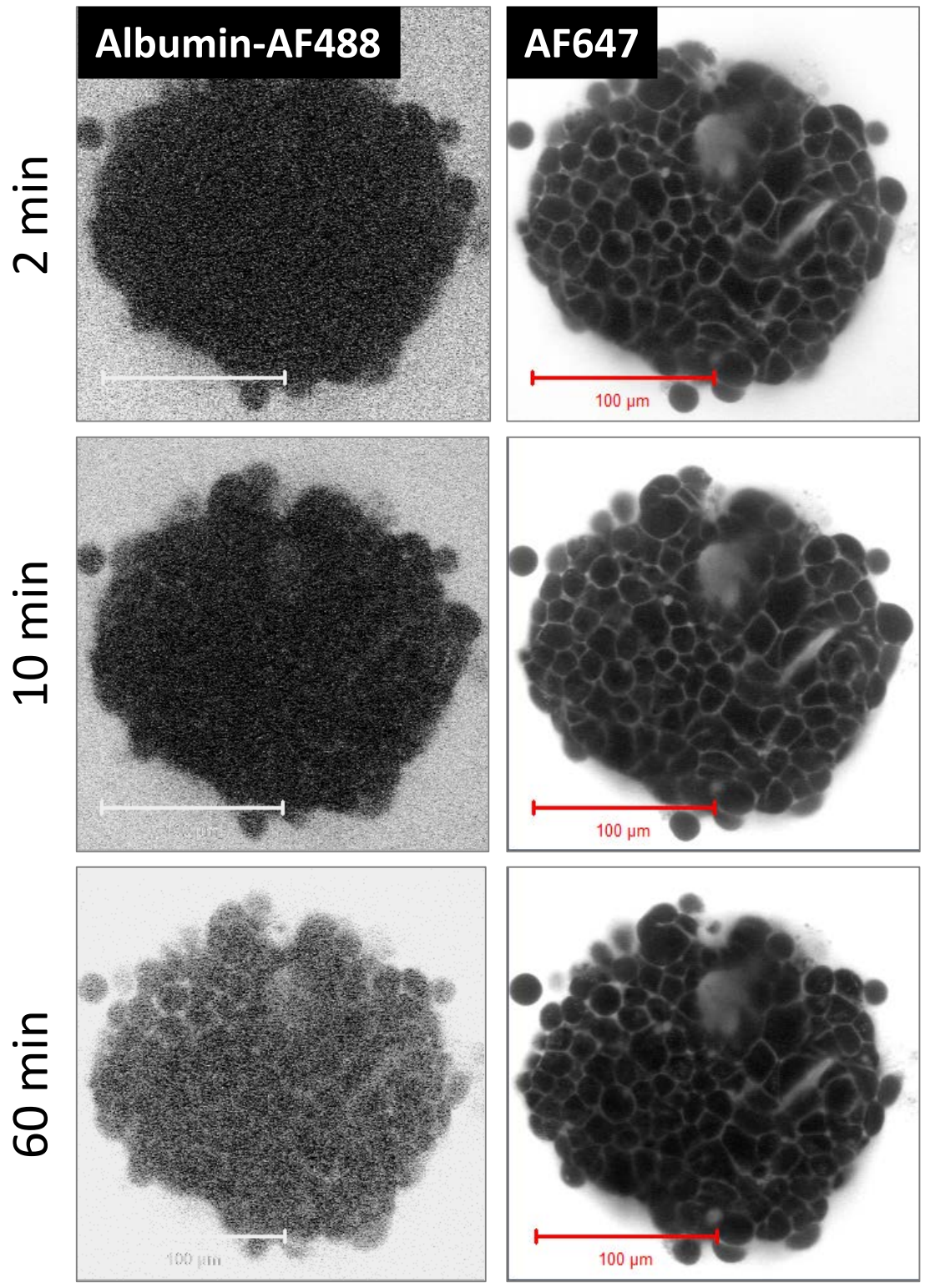
Extended Data Figure 1. The hydrophobicity of the fluorescent dye affects the inflow diffusion rate of the dye into the target spheroid. Dual-color confocal microscopy was performed for HepG2 spheroid NCI using the hydrophilic fluorescent dye AF647 (left) and the hydrophilic fluorescent dye Rhodamine 6G (Rh6G, right). One hour after the addition of the two dyes, compared to AF647, Rh6G fluorescence was observed with marked accumulation only in peripheral cells but little in the core region. Bar; 100 µm. The images were prepared by ZEN software (Zeiss, Jena, Germany).

Extended Data Figure 2



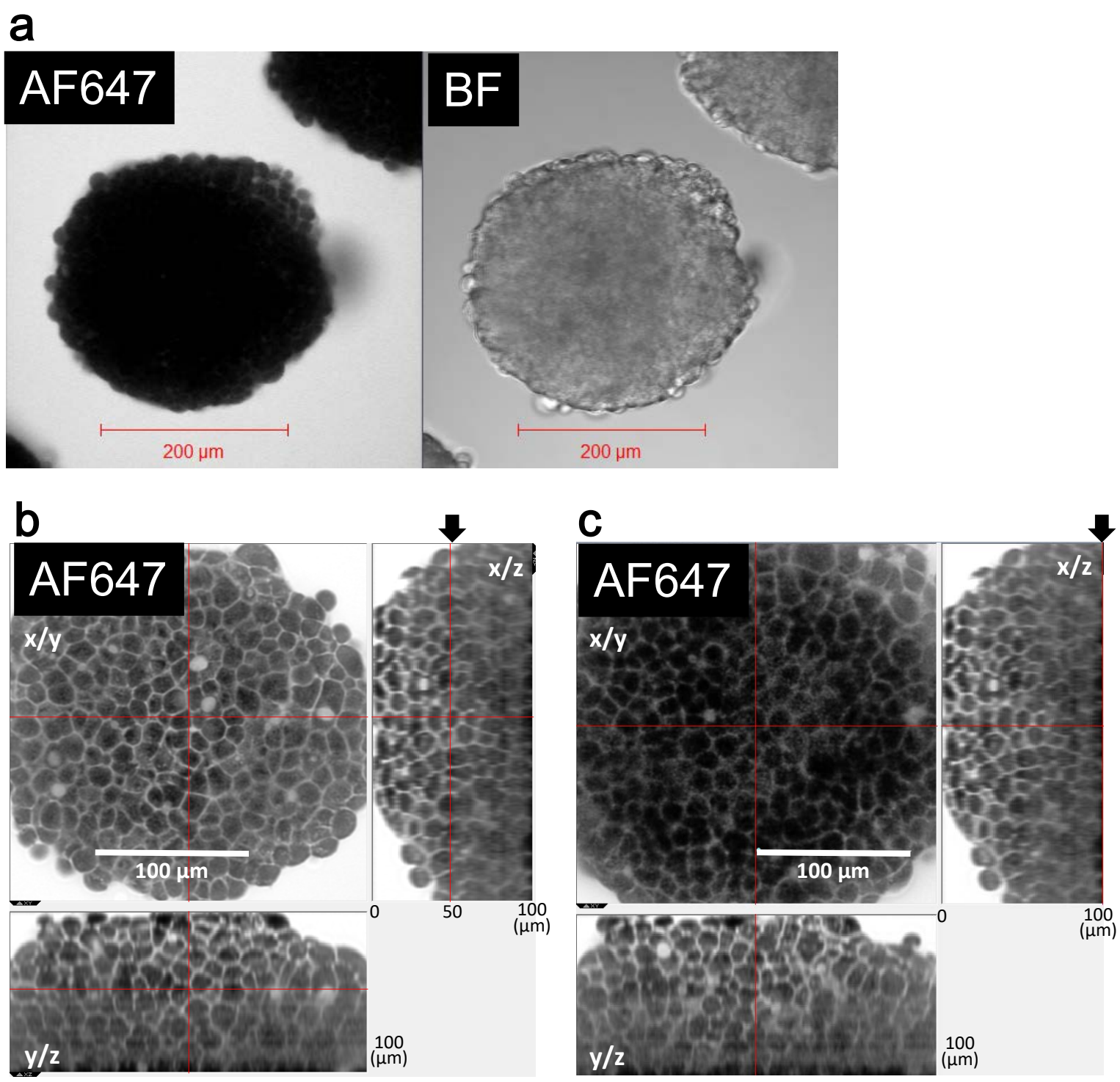
Extended Data Figure 2. NCI accurately visualizes the intercellular space of the spheroid. (a) A dual-color confocal image of a representative HEK293T (HEK) spheroid with transient expression of plasma membrane-targeting AcGFP in the cytosol. NCI with AF647 (left) and AcGFP images (right). Bar: 100 μm. Dashed rectangle: enlarged area in the inset (b). Inset: bright field. Bar: 200 μm. (b) Merged histograms of AcGFP (solid circles) and AF647 (blank triangles) fluorescence intensity along the dashed line in the inset. Inset: Merged image of the indicated dashed square in each image in (a). Square size: 16 × 16 μm. The AcGFP signal (green: intracellular side of the plasma membrane) was located beneath the AF647 signal (white: intercellular). The images were prepared by ZEN software.

Extended Data Figure 3



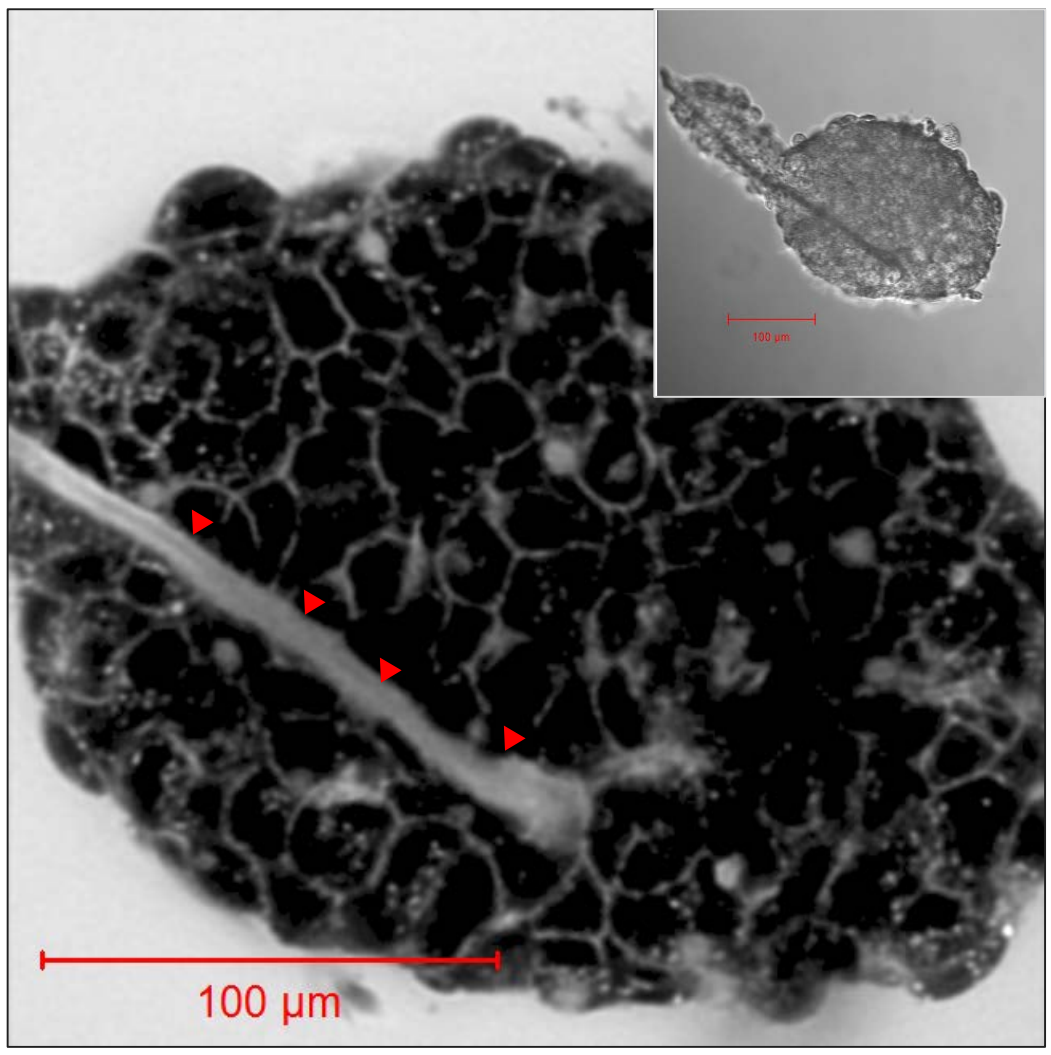
Extended Data Figure 3. The molecular weight of the dye can affect the inflow diffusion rate into the core region of the target spheroids. AF488-conjugated albumin was added to the medium of HepG2 spheroids together with AF647. Dual-color confocal imaging was performed at the same z position of the spheroid over a time course of 2, 10, and 60 min after the addition of dye. Higher molecular weight of dye yielded slower diffusion rates. Bar: 100 µm. The images were prepared by ZEN software.

Extended Data Figure 4



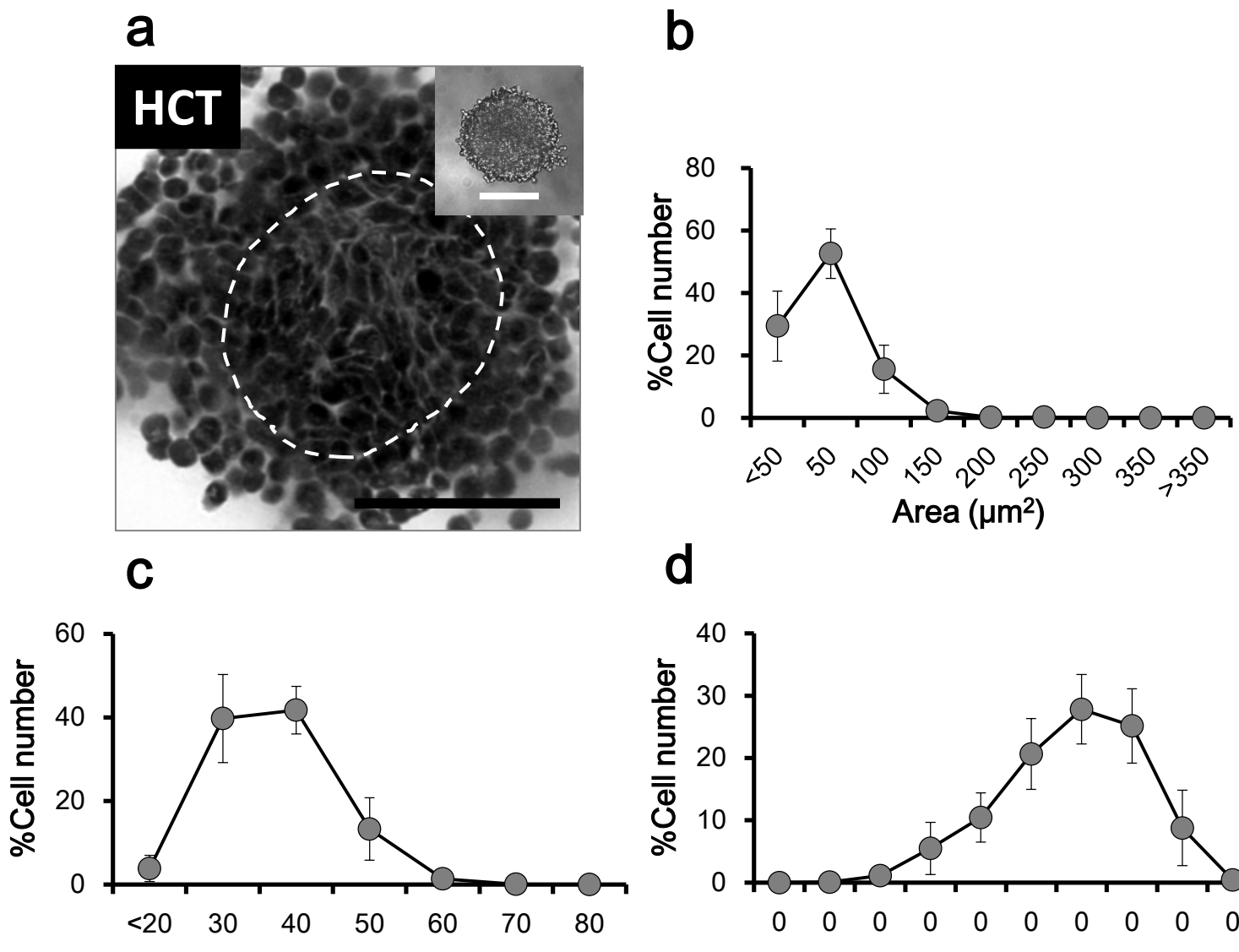
Extended Data Figure 4. NCI visualizes the cells in the deep core region of HepG2 spheroids. (a) Low-magnitude image of the HepG2 spheroid acquired by NCI (left: AF647) and bright field (right: BF). (b, c) Confocal images of the same spheroid in (a) at (b) 50 µm and (c) 100 µm distance from the adhesion plane with the orthogonal images. Bar: 100 µm. Arrow: z position of the x/y plane. Individual cells can be identified by the separation of fluorescent signals in the intercellular space. The images were prepared by ZEN software.

Extended Data Figure 5



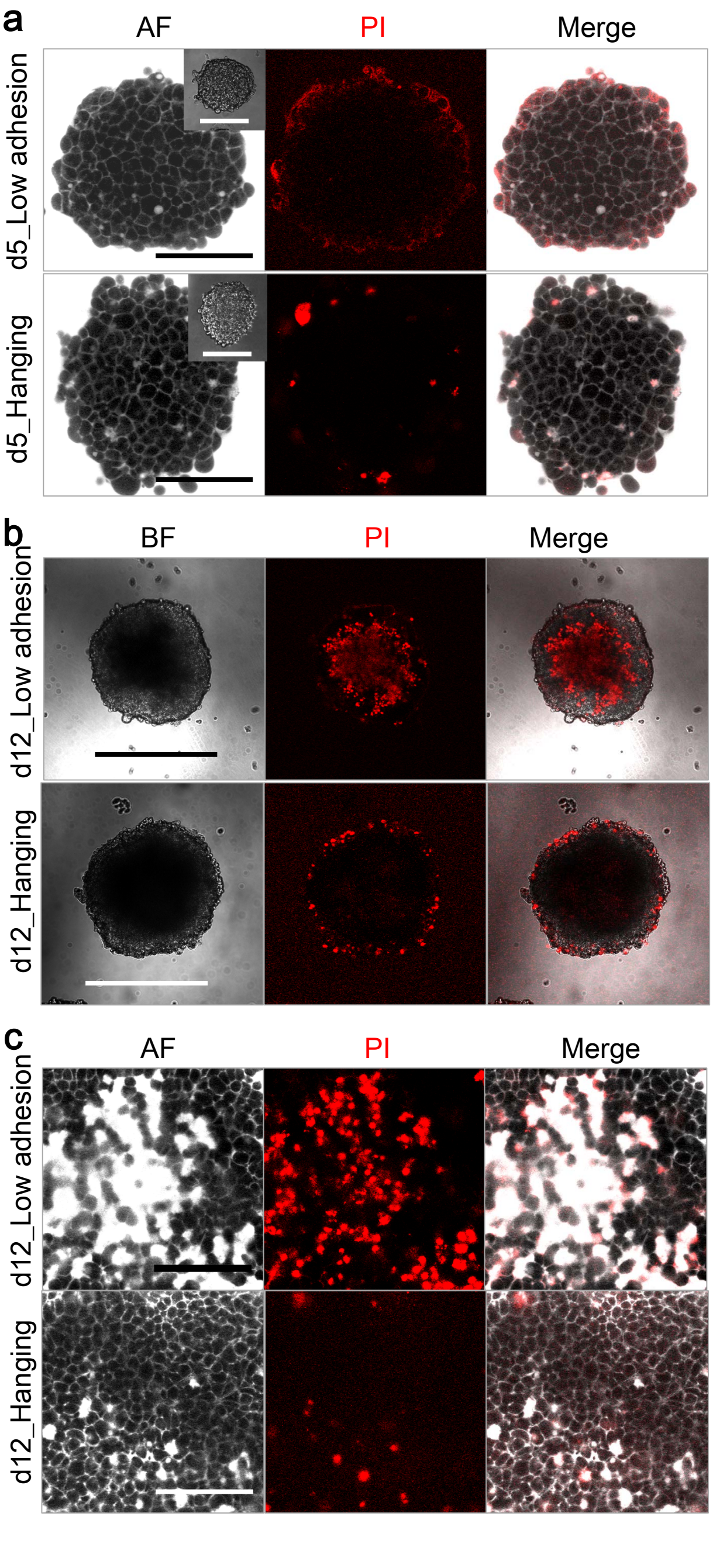
Extended Data Figure 5. NCI can visualize non-cellular insoluble components in spheroids. Representative NCI image of day 5 HepG2 spheroid that embeds insoluble filament component penetrating the dye (arrowhead), which might have originated from debris in the medium. Scale bar: 100 µm. Inset: Low-magnitude image of the spheroid in the bright field. Bar: 100 µm. The images were prepared by ZEN software.

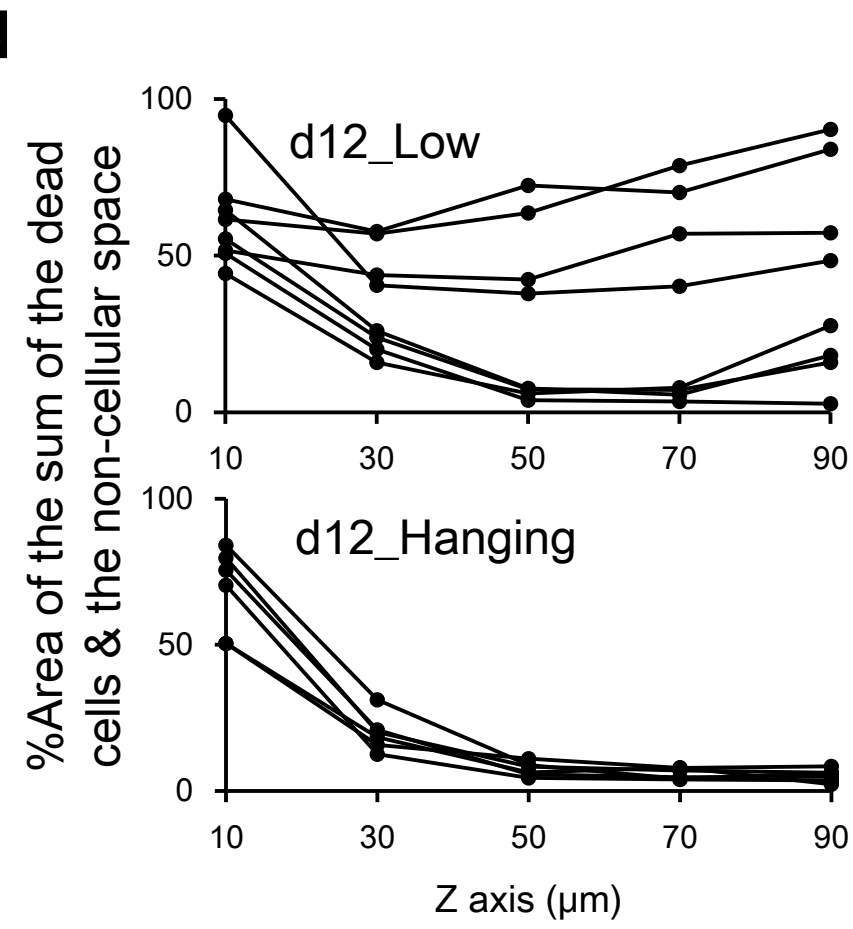
Extended Data Figure 6

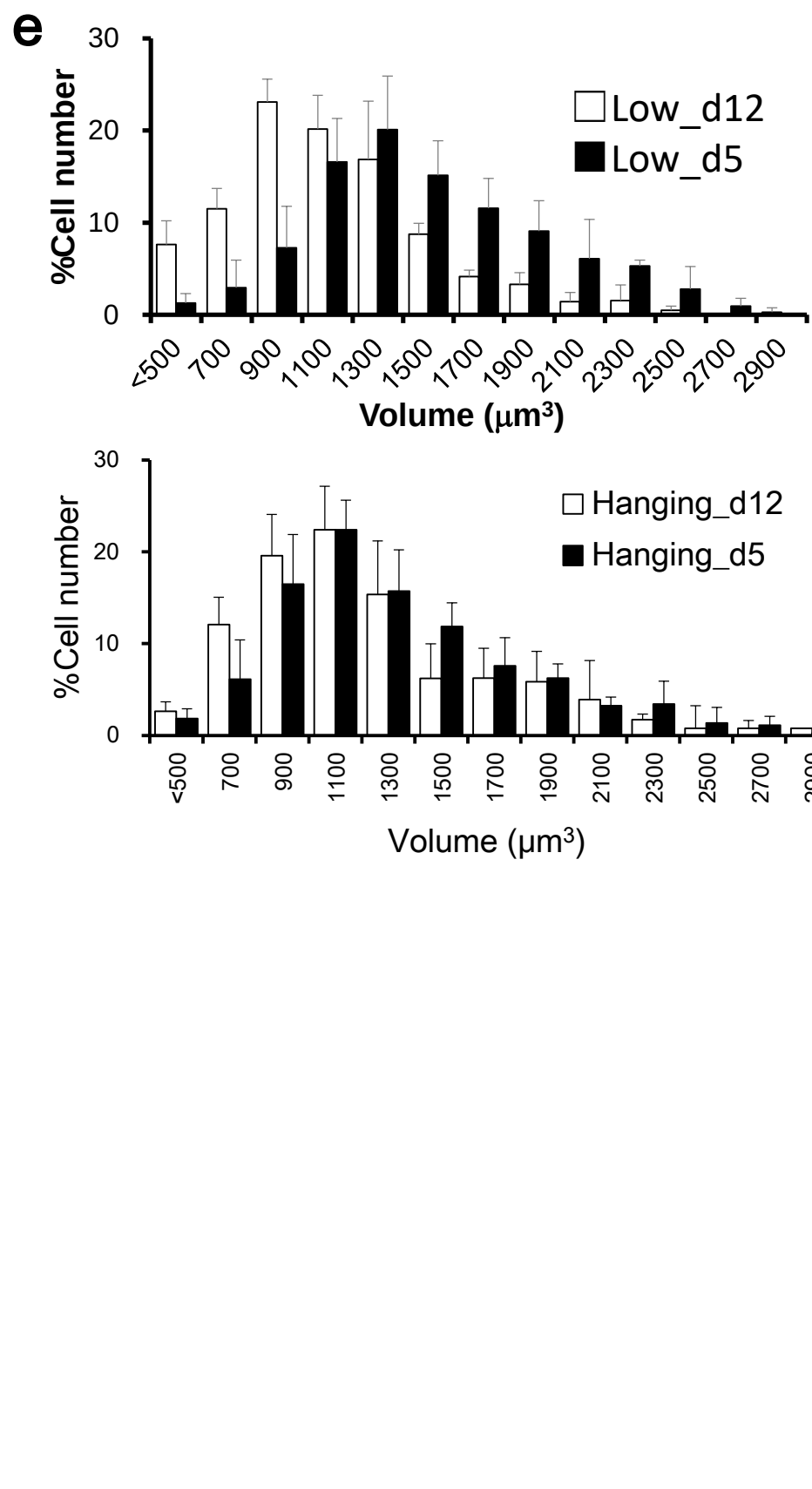


Extended Data Figure 6. NCI of a colon cancer-delivered cell line, HCT-116 (HCT). (a) Representative NCI image of HCT spheroids on day 5 of culture. The dashed line indicates the boundary between the inner tight and outer rough-cell-packaging areas. Bar: 100 μm. Inset: Bright fields. Bar: 200μm. (b-d) Histograms of the individual cellular contours in the spheroid confocal images analyzed by the (b) area, (c) perimeter, and (d) roundness. N = 4. (e) Representative images of the reconstructed 3D structures of individual core cells in HCT spheroids. Each cell is indicated by a different color. Core: core cells; Peri: peripheral cells in (a). (f) Volumetric histogram of the spheroid core cells measured in the same way as in Figure 1i. N = 3. The images of (a) were prepared by ZEN software, and the structural parameters (b-d) , 3D reconstruction of (e) and individual cell volumes of (f) were calculated by Neurolucida (MBF Bioscience, Williston, VT, US).

Extended Data Figure 7



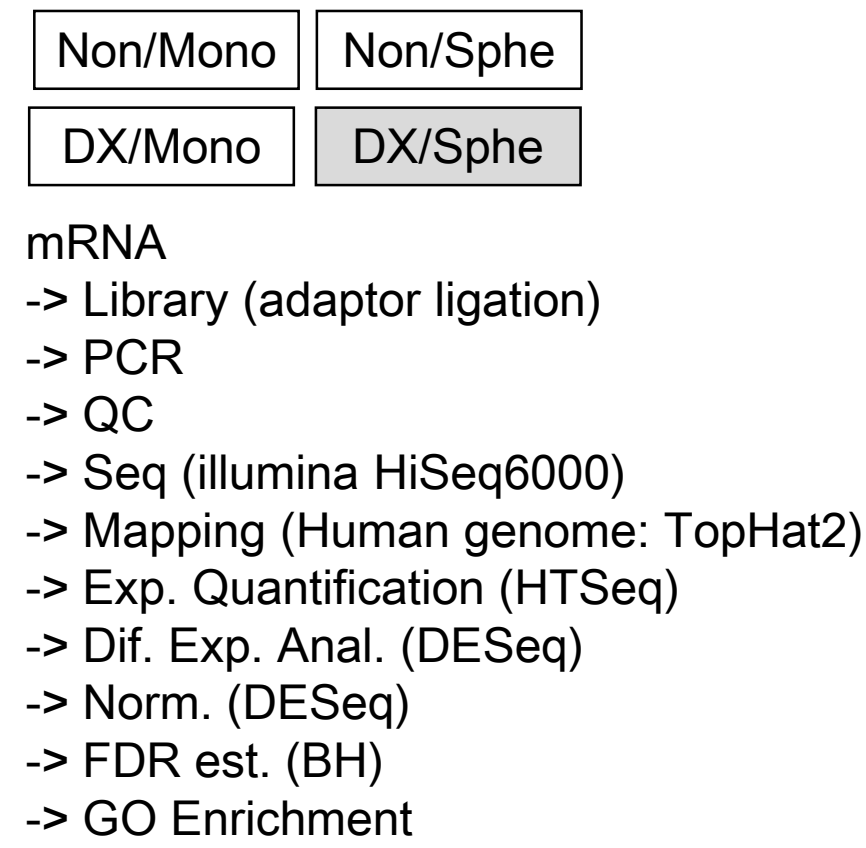




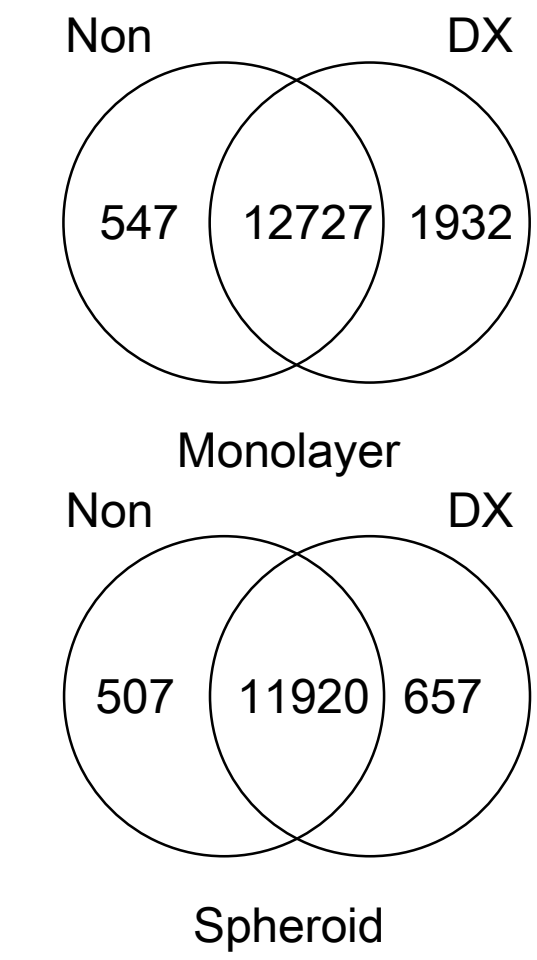
Extended Data Figure 7. NCI shows the difference in structural characteristics and viability of two spheroids cultured under different conditions. (a) HepG2 spheroids cultured for 5 days in a low-adhesion culture plate (upper) and by hanging drop method (lower) visualized by NCI (left column), propidium iodide (PI, middle), and merged (right). Bar: 100 µm. A slight difference was observed between the two spheroids. (b) HepG2 spheroids cultured under the same conditions as in (a) for 12 days. Bright field image (left), confocal image with PI (middle), and merged (right). Scale bar: 500 µm. Clear PI accumulation, which may indicate cell death, was observed in the spheroid core region, which was cultured using a low-adhesion culture plate. (c) Confocal image of the core region of spheroids (b) 100 µm above the adhesion plane. NCI (left column), PI (middle), and merged (right). Scale bar: 100 µm. (d) The content of the area of the sum of dead cells and non-cellular space against the entire scanned area aligned along the z-axis from the adhesion plane to 90 µm. Upper: low-adhesion plate. N = 8. Lower: hanging drop method. N = 6. Each line indicates individual spheroid data. (e) Volumetric histograms of individual core cells of HepG2 spheroids cultured in the low-adhesion culture plate (upper panel) and by hanging drop method (lower panel). The five-day culture data are shown in the solid column, and 12 day culture data are shown in the blank column. The low-adhesion culture plate showed a peak shift in the cell volume histogram. The images of (a-c) were prepared by ZEN software, and calculation of the fluorescence intensity in (d) were by Fiji software (https://imagej.net/Fiji). The volume of the individual cells of (e) were calculated by Neurolucida.

Extended Data Figure 8

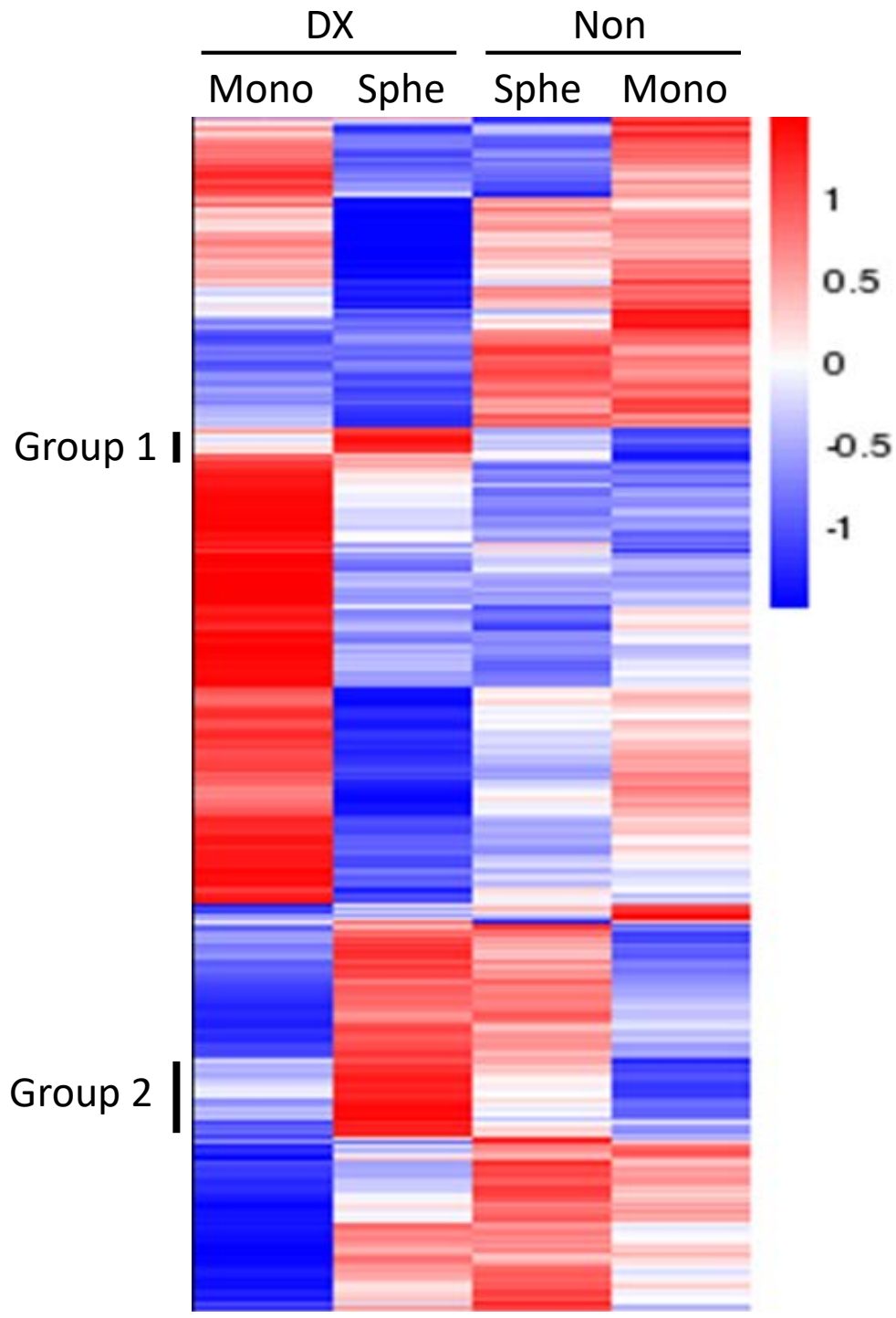
a



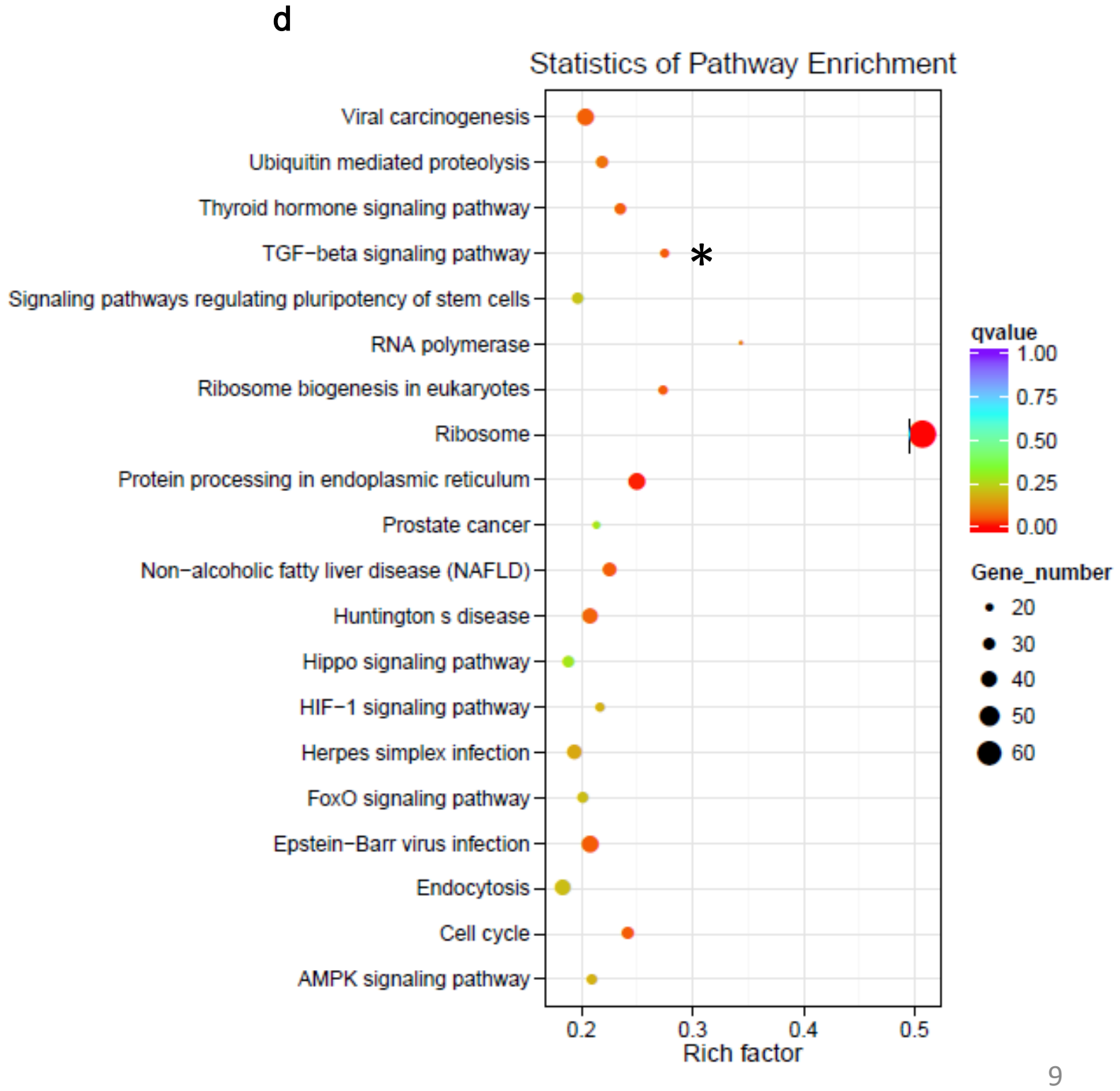
b



c



d



Extended Data Figure 8. Transcriptomic analysis of HepG2 cells under various culture conditions. (a) Schema of transcriptomic analysis. The four conditions in HepG2 cells cultured with monolayer (Mono) or spheroid (Sphe) with/without DX were compared. QC, quality control; FDR, false discovery rate; BH, Benjamini –Hochberg. (b) Venn diagrams representing the number of unique and common genes between the two RNA-seq samples. (c) FPKM cluster analysis (heat map) using the log10 (FPKM+1) value. The color range from red to blue represents the log10 (FPKM+1) value from large to small. Overall, 3,795 of 12,427 genes analyzed were found to be differentially expressed after multiple testing corrections (FDR estimation BH, <0.05). The upregulated gene clusters in DX-treated spheroids were named Group 1 (84 genes) and Group 2 (126 genes), and the list is shown in Extended Data Figure 9 (Group 1) and 10 (Group 2). (d) KEGG enrichment scatter plot of DX enhancement in spheroids. Dot size represents the number of different genes, and color indicates the q-value. Asterisk: TGF-β signaling pathway.

Extended Data Figure 9

Gene ID	Gene Name
ENSG00000137509	prolylcarboxypeptidase(PRCP)
ENSG00000089063	transmembrane protein 230(TMEM230)
ENSG00000156467	ubiquinol-cytochrome c reductase binding protein(UQCRB)
ENSG00000105438	KDEL endoplasmic reticulum protein retention receptor 1(KDELR1)
ENSG00000127184	microRNA 3607(MIR3607)
ENSG00000179222	MAGE family member D1(MAGED1)
ENSG00000099624	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit(ATP5D)
ENSG00000177600	ribosomal protein lateral stalk subunit P2(RPLP2)
ENSG00000065978	Y-box binding protein 1(YBX1)
ENSG00000139644	transmembrane BAX inhibitor motif containing 6(TMBIM6)
ENSG00000111275	aldehyde dehydrogenase 2 family (mitochondrial)(ALDH2)
ENSG00000166794	peptidylprolyl isomerase B(PPIB)
ENSG00000142541	ribosomal protein L13a(RPL13A)
ENSG00000167004	protein disulfide isomerase family A member 3(PDIA3)
ENSG00000170889	ribosomal protein S9(RPS9)
ENSG00000101439	cystatin C(CST3)
ENSG00000163041	H3 histone family member 3A(H3F3A)
ENSG00000169100	solute carrier family 25 member 6(SLC25A6)
ENSG00000105193	ribosomal protein S16(RPS16)
ENSG00000160014	calmodulin 3(CALM3)
ENSG00000109472	carboxypeptidase E(CPE)
ENSG00000130707	argininosuccinate synthase 1(ASS1)
ENSG00000167526	ribosomal protein L13(RPL13)
ENSG00000143870	protein disulfide isomerase family A member 6(PDIA6)
ENSG00000166710	beta-2-microglobulin(B2M)
ENSG00000160213	cystatin B(CSTB)
ENSG00000155368	diazepam binding inhibitor, acyl-CoA binding protein(DBI)
ENSG00000161944	asialoglycoprotein receptor 2(ASGR2)
ENSG00000138207	retinol binding protein 4(RBP4)
ENSG00000197249	serpin family A member 1(SERPINA1)
ENSG00000198931	adenine phosphoribosyltransferase(APRT)
ENSG00000170540	ADP ribosylation factor like GTPase 6 interacting protein 1(ARL6IP1)
ENSG00000115268	ribosomal protein S15(RPS15)
ENSG00000110492	midkine (neurite growth-promoting factor 2)(MDK)
ENSG00000136942	ribosomal protein L35(RPL35)
ENSG00000132386	serpin family F member 1(SERPINF1)
ENSG00000173457	protein phosphatase 1 regulatory inhibitor subunit 14B(PPP1R14B)
ENSG00000233927	ribosomal protein S28(RPS28)
ENSG00000120885	microRNA 6843(MIR6843)
ENSG00000221983	ubiquitin A-52 residue ribosomal protein fusion product 1(UBA52)
ENSG00000162244	ribosomal protein L29(RPL29)
ENSG00000142534	ribosomal protein S11(RPS11)
ENSG00000109861	cathepsin C(CTSC)
ENSG00000149273	ribosomal protein S3(RPS3)
ENSG00000153574	ribose 5-phosphate isomerase A(RPIA)
ENSG00000065154	ornithine aminotransferase(OAT)
ENSG00000112306	ribosomal protein S12(RPS12)
ENSG00000136156	integral membrane protein 2B(ITM2B)
ENSG00000135744	angiotensinogen(AGT)
ENSG00000158104	4-hydroxyphenylpyruvate dioxygenase(HPD)
ENSG00000131143	cytochrome c oxidase subunit 4I1(COX4I1)
ENSG00000117632	microRNA 3917(MIR3917)
ENSG00000135940	cytochrome c oxidase subunit 5B(COX5B)
ENSG00000118137	apolipoprotein A1(APOA1)
ENSG00000116221	mitochondrial ribosomal protein L37(MRPL37)
ENSG00000125827	thioredoxin related transmembrane protein 4(TMX4)
ENSG00000159199	ATP synthase, H+ transporting, mitochondrial Fo complex subunit C1 (subunit 9)(ATP5G1)
ENSG00000186468	ribosomal protein S23(RPS23)
ENSG00000063177	ribosomal protein L18(RPL18)
ENSG00000140988	ribosomal protein S2(RPS2)
ENSG00000147257	glypican 3(GPC3)
ENSG00000129824	ribosomal protein S4, Y-linked 1(RPS4Y1)
ENSG00000147255	immunoglobulin superfamily member 1(IGSF1)
ENSG00000105372	ribosomal protein S19(RPS19)
ENSG00000124299	peptidase D(PEPD)
ENSG00000161203	adaptor related protein complex 2 mu 1 subunit(AP2M1)
ENSG00000093010	microRNA 4761(MIR4761)
ENSG00000137818	ribosomal protein lateral stalk subunit P1(RPLP1)
ENSG00000178445	glycine decarboxylase(GLDC)
ENSG00000163902	ribophorin I(RPN1)
ENSG00000155660	protein disulfide isomerase family A member 4(PDIA4)
ENSG00000083845	ribosomal protein S5(RPS5)
ENSG00000169174	proprotein convertase subtilisin/kexin type 9(PCSK9)
ENSG00000243147	mitochondrial ribosomal protein L33(MRPL33)
ENSG00000244038	dolichyl-diphosphooligosaccharide--protein glycosyltransferase non-catalytic subunit(DDOST)
ENSG00000177556	antioxidant 1 copper chaperone(ATOX1)
ENSG00000175899	alpha-2-macroglobulin(A2M)
ENSG00000167996	ferritin heavy chain 1(FTH1)
ENSG00000206503	major histocompatibility complex, class I, A(HLA-A)
ENSG00000125995	reactive oxygen species modulator 1(ROMO1)
ENSG00000101444	adenosylhomocysteinase(AHCY)
ENSG00000123131	peroxiredoxin 4(PRDX4)
ENSG00000149547	EI24, autophagy associated transmembrane protein(EI24)
ENSG00000105640	ribosomal protein L18a(RPL18A)

Extended Data Figure 9. The contents of Group 1 of the heat map shown in Extended Data Fig. 8b.

Extended Data Figure 10

Gene ID	Gene Name		Gene ID	Gene Name
ENSG00000129562	defender against cell death 1(DAD1)		ENSG00000198034	ribosomal protein S4, X-linked(RPS4X)
ENSG00000134910	STT3A, catalytic subunit of the oligosaccharyltransferase complex(STT3A)		ENSG00000118181	ribosomal protein S25(RPS25)
ENSG00000107537	phytanoyl-CoA 2-hydroxylase(PHYH)		ENSG00000106538	retinoic acid receptor responder 2(RARRES2)
ENSG00000110700	ribosomal protein S13(RPS13)		ENSG00000008988	ribosomal protein S20(RPS20)
ENSG00000116005	prenylcysteine oxidase 1(PCYOX1)		ENSG00000115128	splicing factor 3b subunit 6(SF3B6)
ENSG00000117450	peroxiredoxin 1(PRDX1)		ENSG00000197747	S100 calcium binding protein A10(S100A10)
ENSG00000123349	prefoldin subunit 5(PFDN5)		ENSG00000145425	ribosomal protein S3A(RPS3A)
ENSG00000184500	protein S (alpha)(PROS1)		ENSG00000147604	ribosomal protein L7(RPL7)
ENSG00000143198	microsomal glutathione S-transferase 3(MGST3)		ENSG00000114391	ribosomal protein L24(RPL24)
ENSG00000099937	serpin family D member 1(SERPIND1)		ENSG00000100316	ribosomal protein L3(RPL3)
ENSG00000089220	phosphatidylethanolamine binding protein 1(PEBP1)		ENSG00000185088	ribosomal protein S27 like(RPS27L)
ENSG00000147677	eukaryotic translation initiation factor 3 subunit H(EIF3H)		ENSG00000131828	pyruvate dehydrogenase (lipoamide) alpha 1(PDHA1)
ENSG00000178741	cytochrome c oxidase subunit 5A(COX5A)		ENSG00000143486	eukaryotic translation initiation factor 2D(EIF2D)
ENSG00000126067	proteasome subunit beta 2(PSMB2)		ENSG00000166228	pterin-4 alpha-carbinolamine dehydratase 1(PCBD1)
ENSG00000113648	H2A histone family member Y(H2AFY)		ENSG00000074800	enolase 1(ENO1)
ENSG00000111229	actin related protein 2/3 complex subunit 3(ARPC3)		ENSG00000106049	3-hydroxyisobutyrate dehydrogenase(HIBADH)
ENSG00000132507	eukaryotic translation initiation factor 5A(EIF5A)		ENSG00000172590	mitochondrial ribosomal protein L52(MRPL52)
ENSG00000143183	transmembrane and coiled-coil domains 1(TMCO1)		ENSG00000114902	signal peptidase complex subunit 1(SPCS1)
ENSG00000130208	apolipoprotein C1(APOC1)		ENSG00000142937	ribosomal protein S8(RPS8)
ENSG00000118680	myosin light chain 12B(MYL12B)		ENSG00000196683	translocase of outer mitochondrial membrane 7(TOMM7)
ENSG00000173852	dpy-19 like 1(DPY19L1)		ENSG00000118271	transthyretin(TTR)
ENSG00000116478	histone deacetylase 1(HDAC1)		ENSG00000099783	heterogeneous nuclear ribonucleoprotein M(HNRNPM)
ENSG00000171314	phosphoglycerate mutase 1(PGAM1)		ENSG00000173207	CDC28 protein kinase regulatory subunit 1B(CKS1B)
ENSG00000143621	interleukin enhancer binding factor 2(ILF2)		ENSG00000136930	proteasome subunit beta 7(PSMB7)
ENSG00000168028	ribosomal protein SA(RPSA)		ENSG00000172115	cytochrome c, somatic(CYCS)
ENSG00000114054	propionyl-CoA carboxylase beta subunit(PCCB)		ENSG00000168653	NADH:ubiquinone oxidoreductase subunit S5(NDUFS5)
ENSG00000144713	ribosomal protein L32(RPL32)		ENSG00000117054	acyl-CoA dehydrogenase, C-4 to C-12 straight chain(ACADM)
ENSG00000128928	isovaleryl-CoA dehydrogenase(IVD)		ENSG00000165264	NADH:ubiquinone oxidoreductase subunit B6(NDUFB6)
ENSG00000108298	ribosomal protein L19(RPL19)		ENSG00000100983	glutathione synthetase(GSS)
ENSG00000182551	acireductone dioxygenase 1(ADI1)		ENSG00000126457	protein arginine methyltransferase 1(PRMT1)
ENSG00000142657	phosphogluconate dehydrogenase(PGD)		ENSG00000086232	eukaryotic translation initiation factor 2 alpha kinase 1(EIF2AK1)
ENSG00000081051	alpha fetoprotein(AFP)		ENSG00000175061	LRRC75A antisense RNA 1(LRRC75A-AS1)
ENSG00000164587	ribosomal protein S14(RPS14)		ENSG00000214078	copine 1(CPNE1)
ENSG00000124562	small nuclear ribonucleoprotein polypeptide C(SNRPC)		ENSG00000138326	ribosomal protein S24(RPS24)
ENSG00000204308	ring finger protein 5(RNF5)		ENSG00000119013	NADH:ubiquinone oxidoreductase subunit B3(NDUFB3)
ENSG00000122026	ribosomal protein L21(RPL21)		ENSG00000117601	serpin family C member 1(SERPINC1)
ENSG00000165678	growth hormone inducible transmembrane protein(GHITM)		ENSG00000174547	mitochondrial ribosomal protein L11(MRPL11)
ENSG00000107798	lipase A, lysosomal acid type(LIPA)		ENSG00000106153	coiled-coil-helix-coiled-coil-helix domain containing 2(CHCHD2)
ENSG00000164904	aldehyde dehydrogenase 7 family member A1(ALDH7A1)		ENSG00000131469	ribosomal protein L27(RPL27)
ENSG00000130414	NADH:ubiquinone oxidoreductase subunit A10(NDUFA10)		ENSG00000104325	2,4-dienoyl-CoA reductase 1, mitochondrial(DECR1)
ENSG00000113558	S-phase kinase associated protein 1(SKP1)		ENSG00000110321	eukaryotic translation initiation factor 4 gamma 2(EIF4G2)
ENSG00000145592	ribosomal protein L37(RPL37)		ENSG00000025772	translocase of outer mitochondrial membrane 34(TOMM34)
ENSG00000171560	fibrinogen alpha chain(FGA)		ENSG00000137563	gamma-glutamyl hydrolase(GGH)
ENSG00000196502	sulfotransferase family 1A member 1(SULT1A1)		ENSG00000132432	Sec61 translocon gamma subunit(SEC61G)
ENSG00000058262	Sec61 translocon alpha 1 subunit(SEC61A1)		ENSG00000130255	ribosomal protein L36(RPL36)
ENSG00000164111	annexin A5(ANXA5)		ENSG00000134291	transmembrane protein 106C(TMEM106C)
ENSG00000230989	heat shock factor binding protein 1(HSBP1)		ENSG00000229117	ribosomal protein L41(RPL41)
ENSG00000125868	destrin, actin depolymerizing factor(DSTN)		ENSG00000150779	translocase of inner mitochondrial membrane 8 homolog B(TIMM8B)
ENSG00000117592	peroxiredoxin 6(PRDX6)		ENSG00000051596	THO complex 3(THOC3)
ENSG00000125743	small nuclear ribonucleoprotein D2 polypeptide(SNRPD2)		ENSG00000172809	ribosomal protein L38(RPL38)
ENSG00000182220	ATPase H+ transporting accessory protein 2(ATP6AP2)		ENSG00000102144	phosphoglycerate kinase 1(PGK1)
ENSG00000127922	uncharacterized LOC100506136(LOC100506136)		ENSG00000215021	prohibitin 2(PHB2)
ENSG00000164919	cytochrome c oxidase subunit 6C(COX6C)		ENSG00000140740	ubiquinol-cytochrome c reductase core protein II(UQCRC2)
ENSG00000110955	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide(ATP5B)		ENSG00000188846	ribosomal protein L14(RPL14)
ENSG00000117906	reticulocalbin 2(RCN2)		ENSG00000127884	enoyl-CoA hydratase, short chain 1(ECHS1)
ENSG00000133935	chromosome 14 open reading frame 1(C14orf1)		ENSG00000177156	transaldolase 1(TALDO1)
ENSG00000138029	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit(HADHB)		ENSG00000131238	palmitoyl-protein thioesterase 1(PPT1)
ENSG00000136521	NADH:ubiquinone oxidoreductase subunit B5(NDUFB5)		ENSG00000112977	death associated protein(DAP)
ENSG00000196975	annexin A4(ANXA4)		ENSG00000111640	glyceraldehyde-3-phosphate dehydrogenase(GAPDH)
ENSG00000136888	ATPase H+ transporting V1 subunit G1(ATP6V1G1)		ENSG00000174444	ribosomal protein L4(RPL4)
ENSG00000112695	cytochrome c oxidase subunit 7A2(COX7A2)		ENSG00000173113	tRNA methyltransferase 11-2 homolog (S. cerevisiae)(TRMT112)
ENSG00000116251	ribosomal protein L22(RPL22)		ENSG00000198755	ribosomal protein L10a(RPL10A)
ENSG00000184076	ubiquinol-cytochrome c reductase, complex III subunit X(UQCR10)		ENSG00000228278	orosomucoid 2(ORM2)
ENSG00000143162	cellular repressor of E1A stimulated genes 1(CREG1)		ENSG00000177954	ribosomal protein S27(RPS27)
ENSG00000147400	centrin 2(CETN2)		ENSG00000071082	ribosomal protein L31(RPL31)
ENSG00000147403	ribosomal protein L10(RPL10)		ENSG00000117862	thioredoxin domain containing 12(TXNDC12)
ENSG00000187109	nucleosome assembly protein 1 like 1(NAP1L1)		ENSG00000115561	charged multivesicular body protein 3(CHMP3)
ENSG00000213741	uncharacterized LOC100288910(LOC100288910)		ENSG00000135390	ATP synthase, H+ transporting, mitochondrial Fo complex subunit C2 (subunit 9)(ATP5G2)
ENSG00000075415	solute carrier family 25 member 3(SLC25A3)		ENSG00000099194	stearoyl-CoA desaturase(SCD)
ENSG00000131844	methylcrotonoyl-CoA carboxylase 2(MCCC2)		ENSG00000122406	ribosomal protein L5(RPL5)
ENSG00000069535	monoamine oxidase B(MAOB)		ENSG00000257017	haptoglobin(HP)
ENSG00000103811	cathepsin H(CTSH)		ENSG00000089157	ribosomal protein lateral stalk subunit P0(RPLP0)
ENSG00000211445	glutathione peroxidase 3(GPX3)		ENSG00000184117	nipsnap homolog 1 (C. elegans)(NIPSNAP1)
ENSG00000140319	signal recognition particle 14(SRP14)		ENSG00000125356	NADH:ubiquinone oxidoreductase subunit A1(NDUFA1)
ENSG00000114942	eukaryotic translation elongation factor 1 beta 2(EEF1B2)		ENSG00000115758	ornithine decarboxylase 1(ODC1)
ENSG00000167085	prohibitin(PHB)		ENSG00000106803	Sec61 translocon beta subunit(SEC61B)
ENSG00000104904	ornithine decarboxylase antizyme 1(OAZ1)		ENSG00000156508	eukaryotic translation elongation factor 1 alpha 1(EEF1A1)
ENSG00000044115	catenin alpha 1(CTNNA1)		ENSG00000116288	Parkinsonism associated deglycase(PARK7)
ENSG00000005022	solute carrier family 25 member 5(SLC25A5)		ENSG00000183291	selenoprotein F(SELENOF)
ENSG00000109475	ribosomal protein L34(RPL34)		ENSG00000119326	catenin alpha like 1(CTNNAL1)
ENSG00000070756	microRNA 7705(MIR7705)		ENSG00000173915	microRNA 1307(MIR1307)
ENSG00000181019	NAD(P)H quinone dehydrogenase 1(NQO1)		ENSG00000171858	ribosomal protein S21(RPS21)
ENSG00000087086	ferritin light chain(FTL)		ENSG00000164096	chromosome 4 open reading frame 3(C4orf3)
ENSG00000103363	transcription elongation factor B subunit 2(TCEB2)		ENSG00000125691	ribosomal protein L23(RPL23)
ENSG00000002549	leucine aminopeptidase 3(LAP3)		ENSG00000130005	guanidinoacetate N-methyltransferase(GAMT)
ENSG00000214026	mitochondrial ribosomal protein L23(MRPL23)		ENSG00000132541	reactive intermediate imine deaminase A homolog(RIDA)
ENSG00000197043	annexin A6(ANXA6)		ENSG00000005893	lysosomal associated membrane protein 2(LAMP2)
ENSG00000068697	lysosomal protein transmembrane 4 alpha(LAPTM4A)		ENSG00000133872	store-operated calcium entry associated regulatory factor(SARAF)
ENSG00000111666	choline phosphotransferase 1(CHPT1)		ENSG00000138760	scavenger receptor class B member 2(SCARB2)
ENSG00000111669	triosephosphate isomerase 1(TPI1)		ENSG00000143549	tropomyosin 3(TPM3)
ENSG00000072274	transferrin receptor(TFRC)		ENSG00000231500	ribosomal protein S18(RPS18)
ENSG00000111786	serine and arginine rich splicing factor 9(SRSF9)		ENSG00000182199	serine hydroxymethyltransferase 2(SHMT2)
ENSG00000135776	ATP binding cassette subfamily B member 10(ABCB10)		ENSG00000051620	heme binding protein 2(HEBP2)
ENSG00000133112	tumor protein, translationally-controlled 1(TPT1)		ENSG00000135929	cytochrome P450 family 27 subfamily A member 1(CYP27A1)
ENSG00000165475	crystallin lambda 1(CRYL1)		ENSG00000204628	receptor for activated C kinase 1(RACK1)
ENSG00000197958	ribosomal protein L12(RPL12)		ENSG00000182899	ribosomal protein L35a(RPL35A)
ENSG00000060971	acetyl-CoA acyltransferase 1(ACAA1)		ENSG00000092010	proteasome activator subunit 1(PSME1)
ENSG00000137154	ribosomal protein S6(RPS6)		ENSG00000172172	mitochondrial ribosomal protein L13(MRPL13)
ENSG00000100897	DDB1 and CUL4 associated factor 11(DCAF11)		ENSG00000104763	N-acylsphingosine amidohydrolase 1(ASAH1)
ENSG00000166441	ribosomal protein L27a(RPL27A)		ENSG00000075239	acetyl-CoA acetyltransferase 1(ACAT1)
ENSG00000143947	ribosomal protein S27a(RPS27A)		ENSG00000196262	peptidylprolyl isomerase A(PPIA)
ENSG00000125970	RALY heterogeneous nuclear ribonucleoprotein(RALY)		ENSG00000176890	thymidylate synthetase(TYMS)
ENSG00000175390	eukaryotic translation initiation factor 3 subunit F(EIF3F)		ENSG00000111058	acyl-CoA synthetase short-chain family member 3(ACSS3)
ENSG00000125971	dynein light chain roadblock-type 1(DYNLRB1)		ENSG00000130332	LSM7 homolog, U6 small nuclear RNA and mRNA degradation associated(LSM7)
ENSG00000134779	tubulin polyglutamylase complex subunit 2(TPGS2)		ENSG00000254999	BRICK1, SCAR/WAVE actin nucleating complex subunit(BRK1)
ENSG00000124172	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit(ATP5E)		ENSG00000189056	reelin(RELN)
ENSG00000135624	chaperonin containing TCP1 subunit 7(CCT7)		ENSG00000084754	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit(HADHA)
ENSG00000173660	ubiquinol-cytochrome c reductase hinge protein(UQCRH)		ENSG00000137880	GTP cyclohydrolase I feedback regulator(GCHFR)
ENSG00000112667	2'-deoxynucleoside 5'-phosphate N-hydrolase 1(DNPH1)		ENSG00000169567	histidine triad nucleotide binding protein 1(HINT1)
ENSG00000175166	proteasome 26S subunit, non-ATPase 2(PSMD2)		ENSG00000229314	orosomucoid 1(ORM1)
ENSG00000110245	apolipoprotein C3(APOC3)		ENSG00000090621	poly(A) binding protein cytoplasmic 4(PABPC4)
ENSG00000197635	dipeptidyl peptidase 4(DPP4)		ENSG00000163586	fatty acid binding protein 1(FABP1)
ENSG00000197756	ribosomal protein L37a(RPL37A)		ENSG00000163466	actin related protein 2/3 complex subunit 2(ARPC2)
ENSG00000151552	quinoid dihydropteridine reductase(QDPR)		ENSG00000148303	ribosomal protein L7a(RPL7A)
ENSG00000198848	carboxylesterase 1(CES1)		ENSG00000077549	capping actin protein of muscle Z-line beta subunit(CAPZB)
ENSG00000142168	superoxide dismutase 1, soluble(SOD1)		ENSG00000064115	transmembrane 7 superfamily member 3(TM7SF3)
ENSG00000196419	X-ray repair cross complementing 6(XRCC6)		ENSG00000175334	barrier to autointegration factor 1(BANF1)
ENSG00000165487	mitochondrial calcium uptake 2(MICU2)		ENSG00000171530	tubulin folding cofactor A(TBCA)
ENSG00000088832	microRNA 6869(MIR6869)		ENSG00000140374	electron transfer flavoprotein alpha subunit(ETFA)
ENSG00000089009	ribosomal protein L6(RPL6)		ENSG00000203875	small nucleolar RNA host gene 5(SNHG5)
ENSG00000143252	succinate dehydrogenase complex subunit C(SDHC)		ENSG00000084623	eukaryotic translation initiation factor 3 subunit I(EIF3I)
ENSG00000149476	triokinase and FMN cyclase(TKFC)		ENSG00000189043	NDUFA4, mitochondrial complex associated(NDUFA4)
ENSG00000105202	fibrillarin(FBL)		ENSG00000168243	G protein subunit gamma 4(GNG4)
ENSG00000047634	sex comb on midleg-like 1 (Drosophila)(SCML1)		ENSG00000006042	transmembrane protein 98(TMEM98)
ENSG00000102172	spermine synthase(SMS)		ENSG00000142676	ribosomal protein L11(RPL11)
ENSG00000172340	succinate-CoA ligase GDP-forming beta subunit(SUCLG2)		ENSG00000107223	endothelial differentiation related factor 1(EDF1)

Extended Data Figure 11

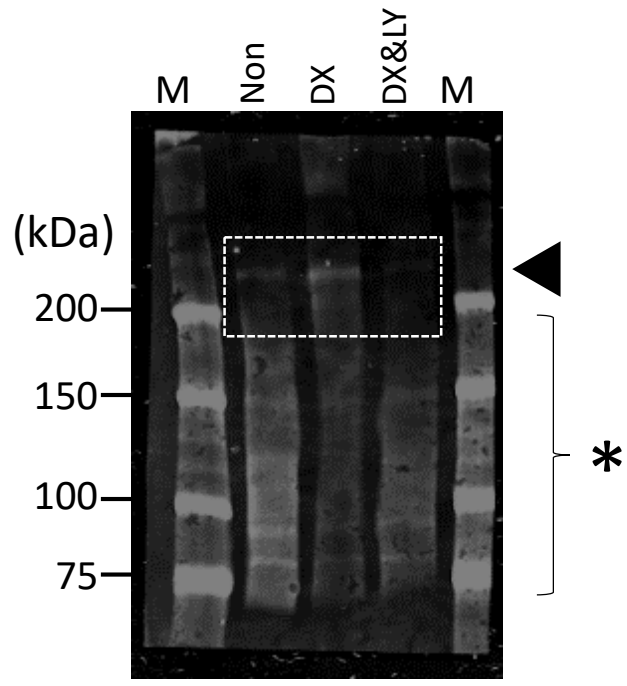
		FPKM			
	Gene	Non_Sphe	Dx_Sphe	Non_Mono	Dx_Mono
Integrin	ITGA1	13	16	19	19
	ITGA2	10	4	22	10
	ITGA2B	0	1	0	13
	ITGA3	1	1	3	6
	ITGA4	0	0	0	0
	ITGA5	0	0	0	1
	ITGA6	33	27	26	24
	ITGA7	2	1	3	9
	ITGA8	0	0	0	0
	ITGA9	0	0	0	1
	ITGA10	0	0	2	2
	ITGA11	0	0	0	0
	ITGAD	0	0	0	0
	ITGAE	22	28	20	18
	ITGAL	7	5	10	7
	ITGAM	0	0	0	6
	ITGAV	47	50	85	55
	ITGAX	0	0	0	0
	ITGB1	328	481	389	378
	ITGB1BP1	80	85	53	52
	ITGB1BP2	0	0	1	0
	ITGB1P1	0	0	0	1
	ITGB2	6	12	20	20
	ITGB3	0	0	0	0
	ITGB3	0	0	0	0
	ITGB3BP	24	24	18	8
	ITGB4	2	2	4	13
	ITGB5	69	82	142	130
	ITGB6	0	0	0	0
	ITGB7	1	0	1	3
	ITGB8	0	0	0	0
	ITGBL1	0	0	0	0

		FPKM			
	Gene	Non_Sphe	Dx_Sphe	Non_Mono	Dx_Mono
Collagen	COL1A1	0	0	1	3
	COL1A2	0	0	0	1
	COL2A1	171	312	157	288
	COL4A1	0	0	0	0
	COL4A2	1	0	1	1
	COL4A3	0	0	0	0
	COL4A4	0	0	0	0
	COL4A5	1	1	0	1
	COL4A6	0	0	0	0
	COL5A1	0	1	1	1
	COL5A2	1	1	1	2
	COL5A3	0	0	0	0
	COL6A1	3	5	5	23
	COL6A2	0	0	0	2
	COL6A5	0	0	0	0
	COL6A6	0	0	0	0
	COL7A1	15	21	73	97
	COL8A1	0	0	0	0
	COL8A2	0	0	0	1
	COL9A1	0	0	0	1
	COL9A3	84	141	293	437
	COL9A2	12	16	45	76
	COL10A1	0	0	0	0
	COL11A1	0	0	0	0
	COL11A2	0	0	1	3
	COL12A1	0	0	0	0
	COL13A1	0	0	0	0
	COL14A1	0	0	0	1
	COL15A1	0	0	0	0
	COL16A1	7	8	7	11
	COL17A1	0	0	0	1
	COL18A1	55	100	175	200
	COL19A1	0	0	0	0
	COL20A1	0	0	0	3
	COL21A1	78	116	97	68
	COL22A1	0	0	0	0
	COL23A1	0	0	0	0
	COL24A1	0	0	0	0
	COL25A1	0	0	0	0
	COL27A1	55	57	144	248
	COL28A1	0	0	0	1

		FPKM			
	Gene	Non_Sphe	Dx_Sphe	Non_Mono	Dx_Mono
Proteoglycan	CSPG4P4Y	0	0	0	0
	CSPG4	0	0	0	1
	CSPG4P1Y	0	0	0	0
	CSPG4P2Y	0	0	0	0
	CSPG4P3Y	0	0	0	0
	CSPG4P5	0	0	0	0
	CSPG5	0	1	0	5
	HAPLN1	0	0	0	0
	HAPLN2	0	0	0	3
	HAPLN3	0	0	0	0
	HAPLN4	0	0	0	0
	HSPG2	30	54	148	320
	IMPG1	0	0	0	0
	IMPG2	0	0	0	0
	IMPG2	0	0	0	0
	LEPRE1	61	96	120	178
	PRG2	0	0	0	0
	PRG3	0	0	0	0
	PRG4	0	0	0	0
	SPOCK1	0	0	0	0
	SPOCK2	34	55	192	311
	SPOCK3	0	0	0	0
	TSKU	42	5	44	50

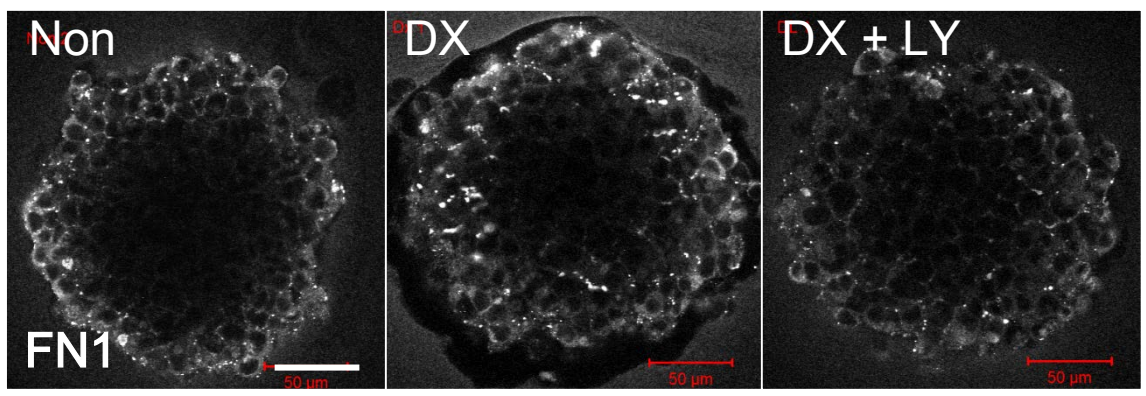
Extended Data Figure 11. Systematic comparison of the transcription mass (FPKM) of the ECM-related gene series expressed in HepG2 cells under several culture conditions. All data were obtained from RNA-seq results.

Extended Data Figure 12



Extended Data Figure 12. The whole image of immunoblotting analysis of fibronectin expression shown in Fig. 4g. Arrowhead: non-disassembled FN (285 kDa). Asterisk: a putative disassembled FN. Dashed rectangle: area shown in Fig. 4g. The image was prepared by Odyssey system (LI-COR, Lincoln, NB, USA).

Extended Data Figure 13



Extended Data Figure 13. Immunofluorescence of fibronectin in HepG2 spheroids after drug treatment. Day 5 HepG2 spheroids of non-treated or treated with DX or with DX and the TGFR inhibitor (LY) were embedded in the Smear Gell, fixed, permeabilized with detergent, and immunostained with rat anti-FN1 antibody and fluorescence-conjugated secondary antibody. Images were acquired using the confocal microscopy. Scale bar: 50 µm. .The images were prepared by ZEN software (Zeiss, Jena, Germany),