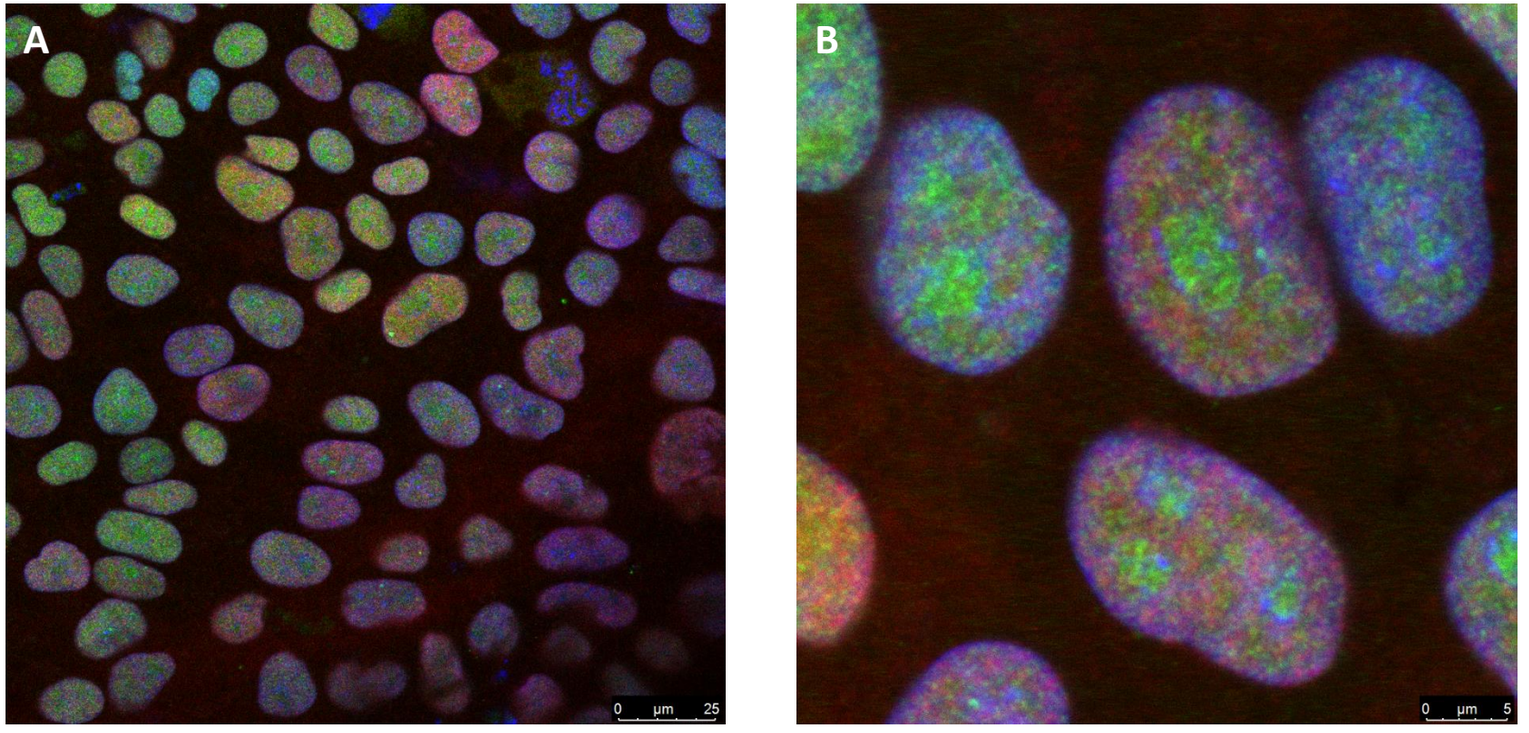
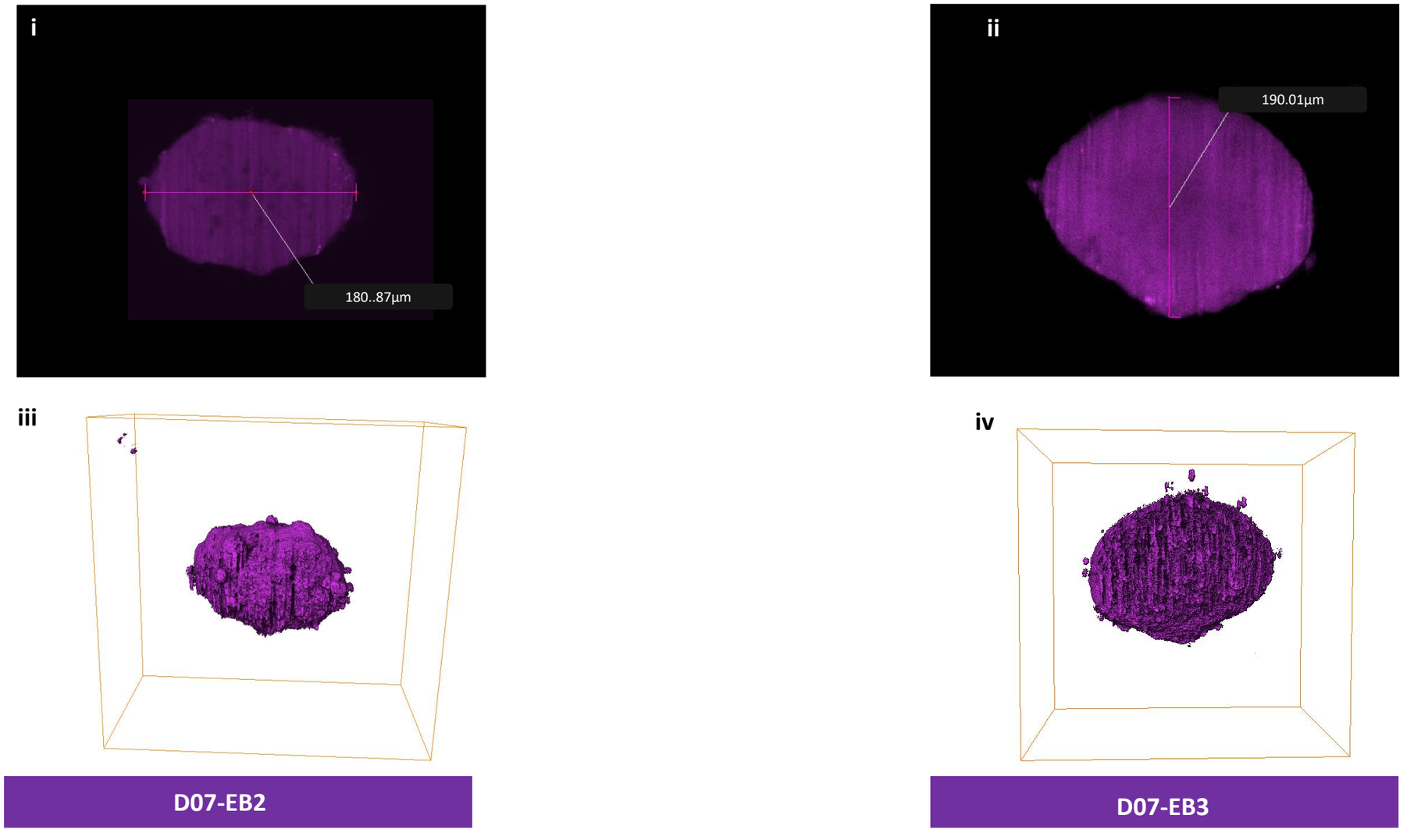
Supplementary figure 1

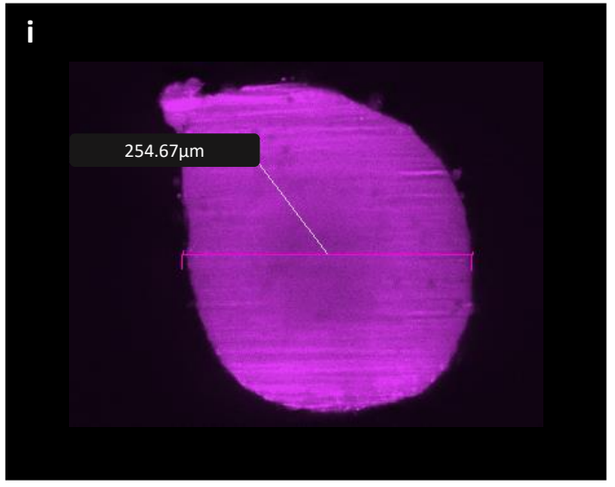


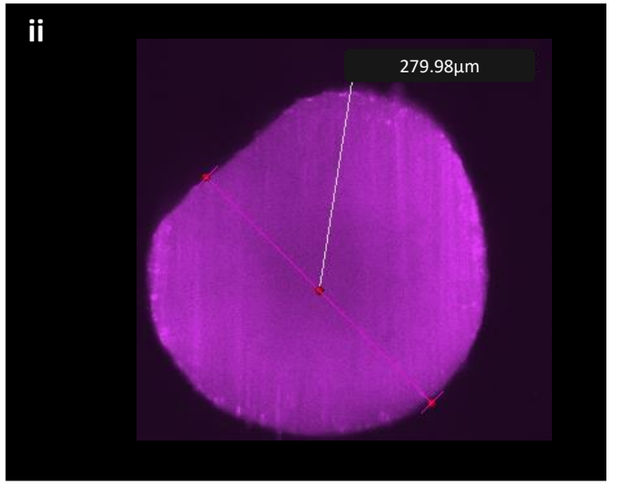
Supplementary Figure 1. Immunofluorescence staining of iPSCs using pluripotent markers. A) Immunofluorescence staining of pluripotent markers SOX2 (Red), NANOG (Green) and counterstain is with DAPI (blue), 20 x magnification. B) 40 x magnification showing the same as A. Scale bars are 25 µm for A and 5 µm for B.

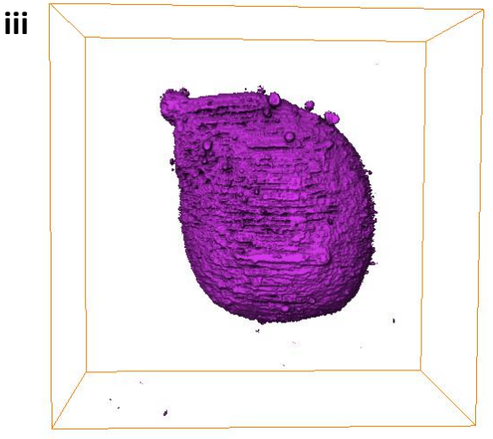
Supplementary figure 2

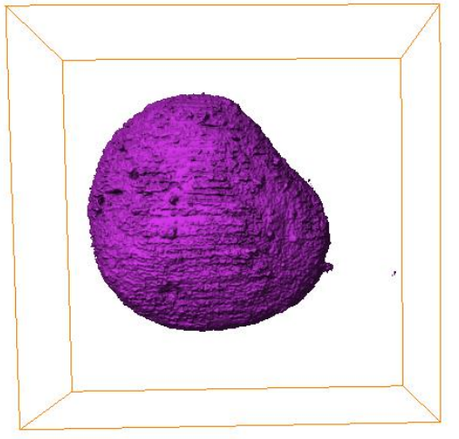


Supplementary Figure 2A. Characterization of day 7 EBs. (i-ii) Diameter measurements of EBs (iii-iv) Volume rendering of EBs. Bounding box size for each EB (iii-iv) is given in Supplementary Table 1.





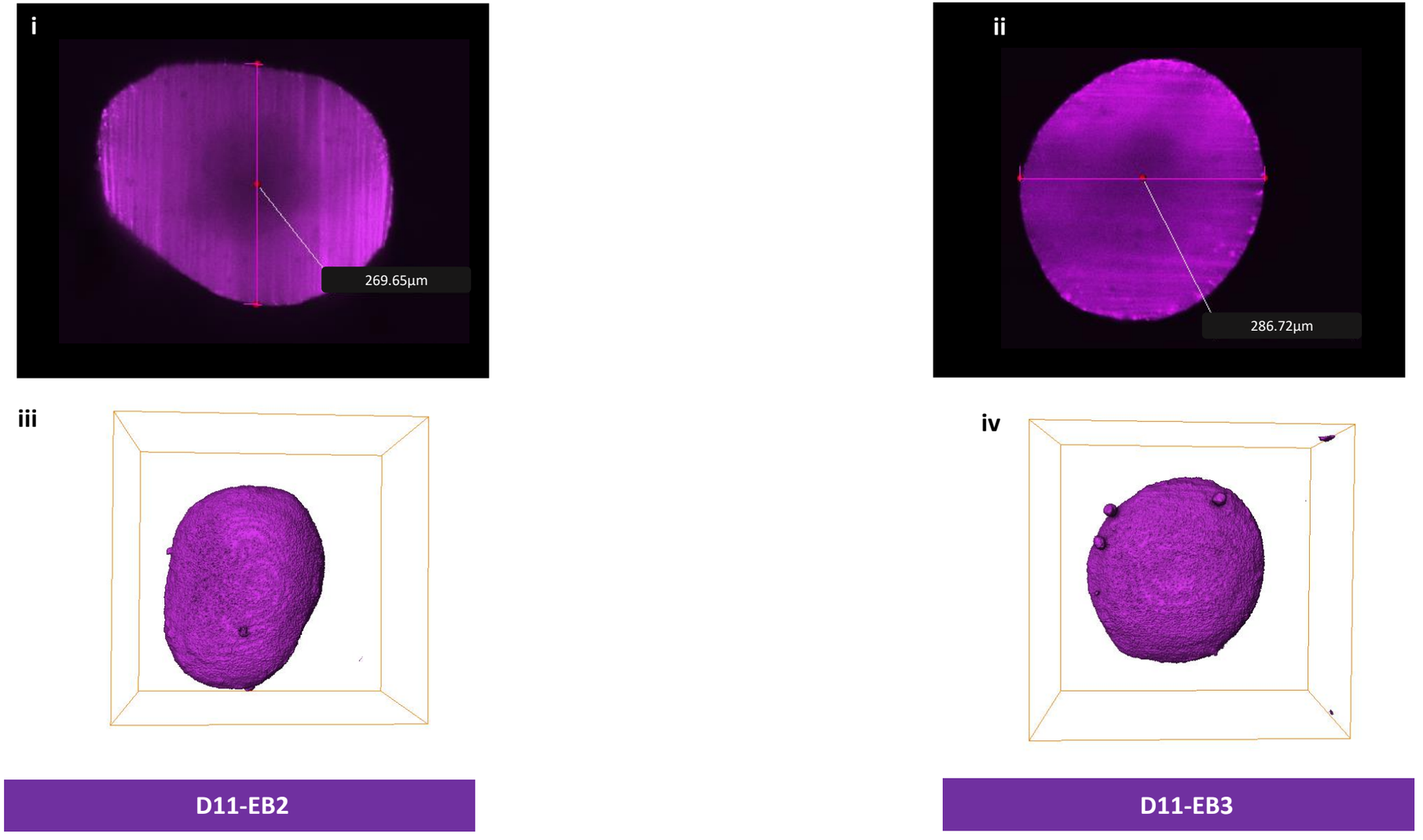




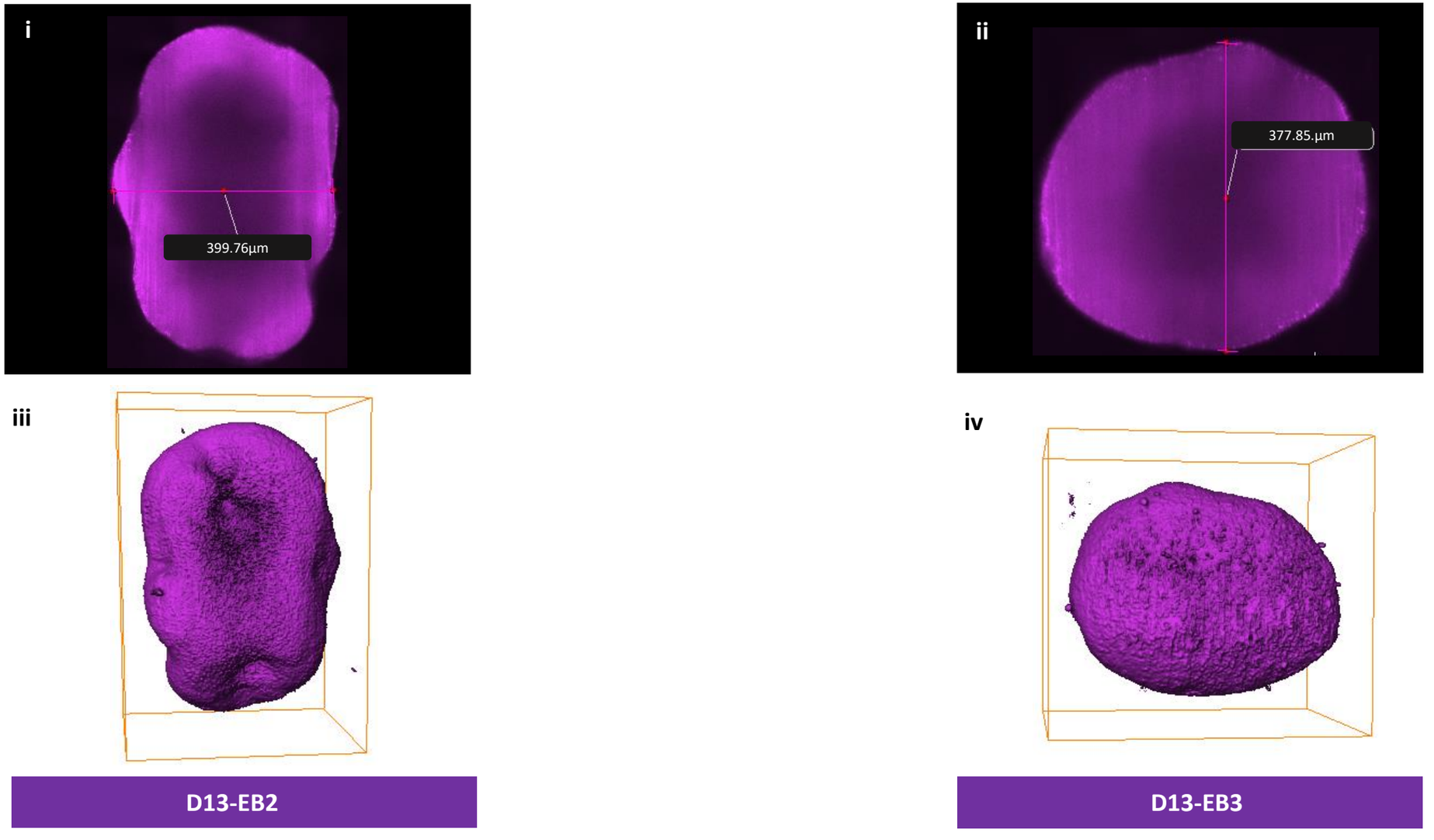
D10-EB2

D10-EB3

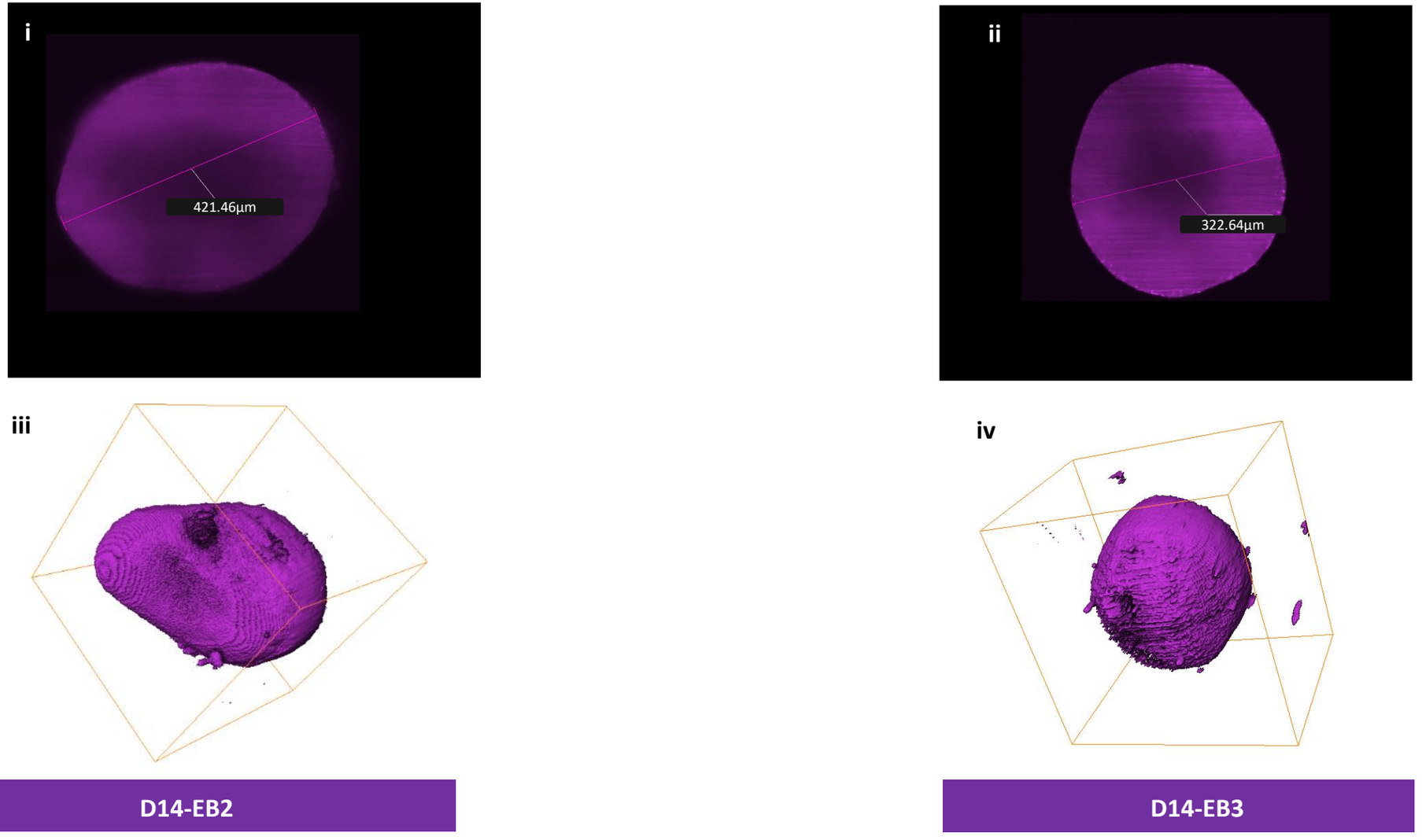
Supplementary Figure 2B. Characterization of day 10 EBs. (i-ii) Diameter measurements of EBs (iii-iv) Volume rendering of EBs. Bounding box size for each EB (iii-iv) is given in Supplementary Table 1.



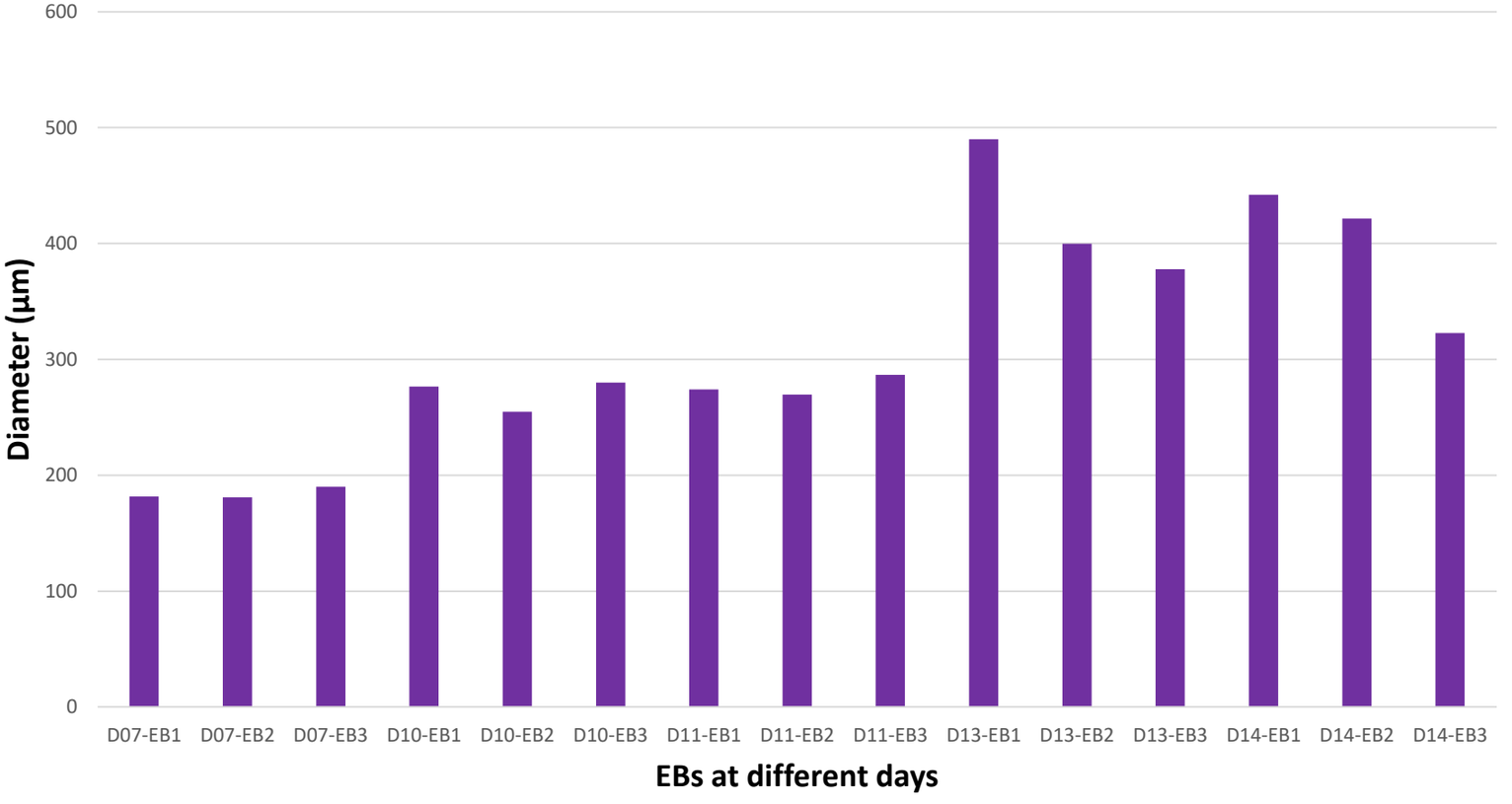
Supplementary Figure 2C. Characterization of day 11 EBs. (i-ii) Diameter measurements of EBs (iii-iv) Volume rendering of EBs. Bounding box size for each EB (iii-iv) is given in Supplementary Table 1.



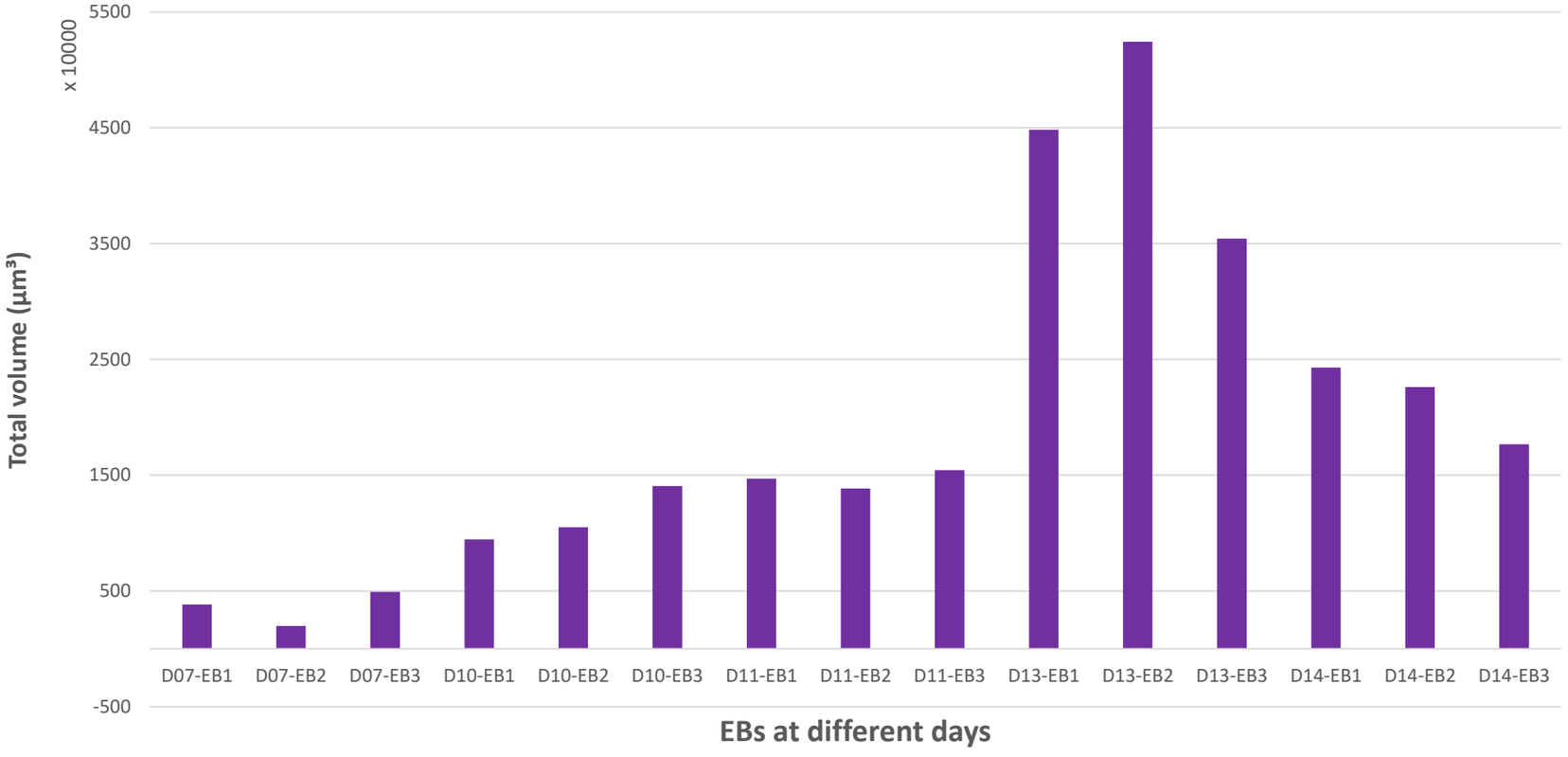
Supplementary Figure 2D. Characterization of day 13 EBs. (i-ii) Diameter measurements of EBs (iii-iv) Volume rendering of EBs. Bounding box size for each EB (iii-iv) is given in Supplementary Table 1.



Supplementary Figure 2E. Characterization of day 14 EBs. (i-ii) Diameter measurements of EBs (iii-iv) Volume rendering of EBs. Bounding box size for each EB (iii-iv) is given in Supplementary Table 1.

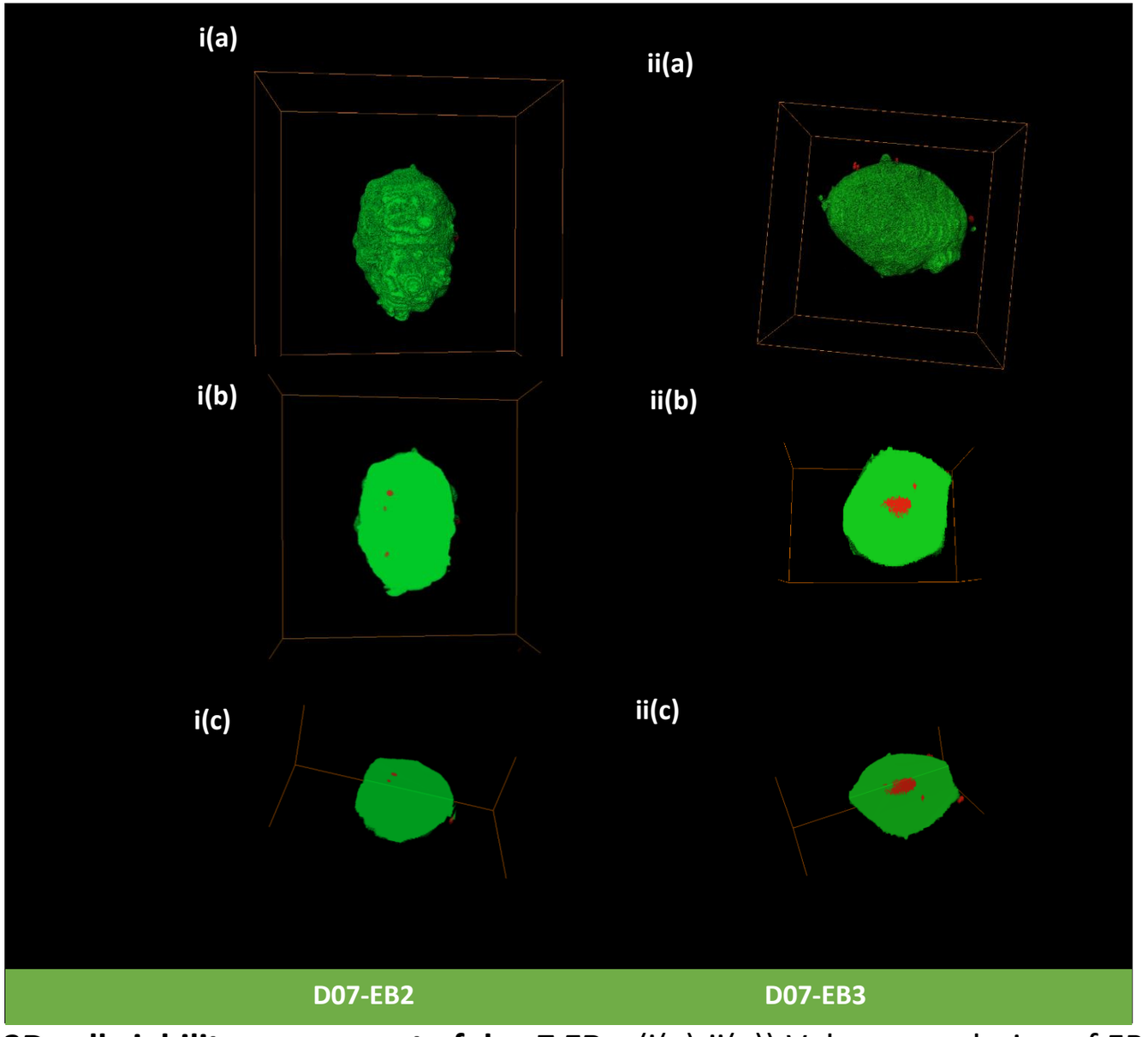


Supplementary Figure 2F. Diameter analysis of individual EBs. Graph showing the diameter of individual EBs from different days during growth.

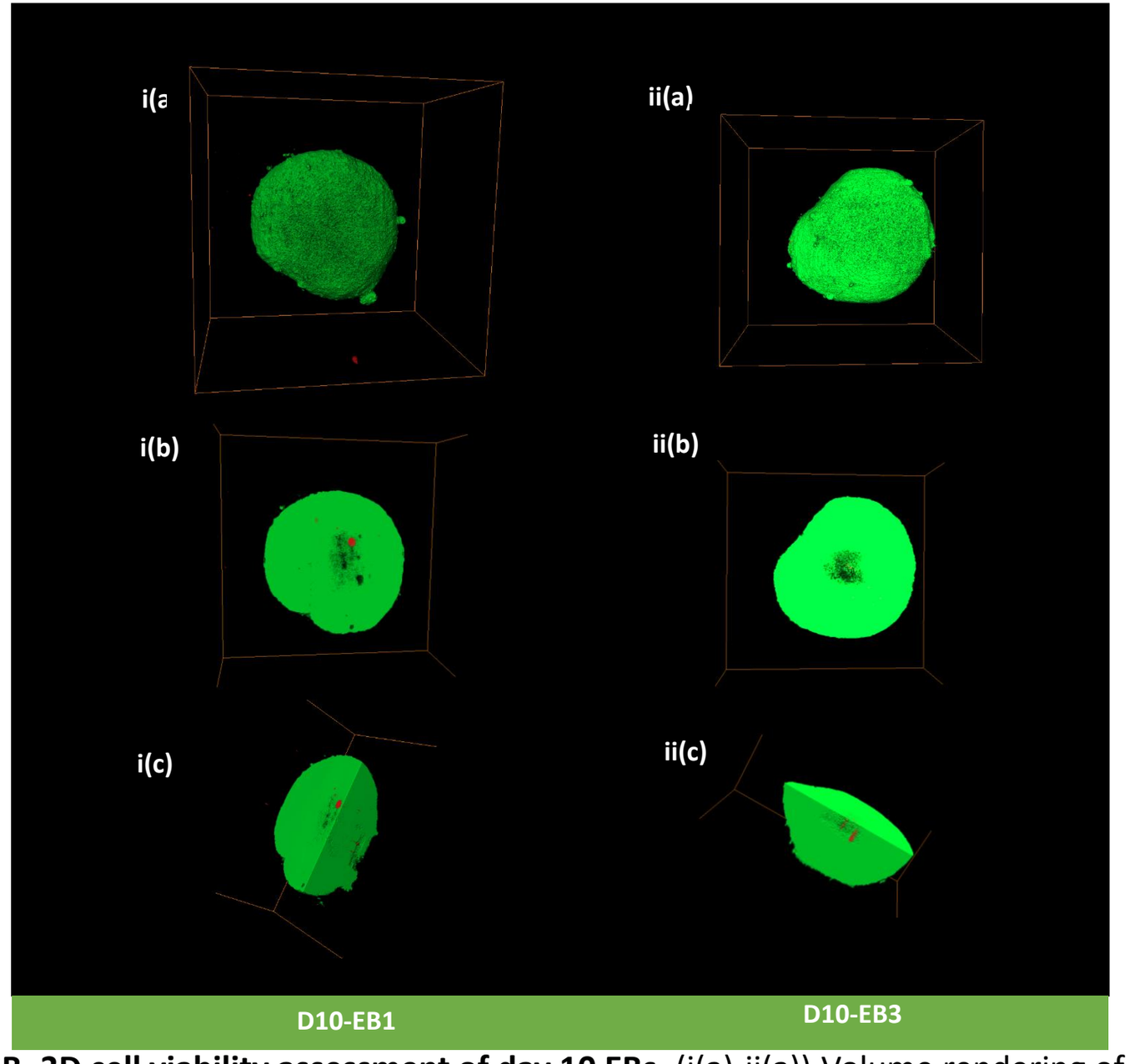


Supplementary Figure 2H. Volume analysis of individual EBs. Graph showing the volume of individual EBs from different days during growth.

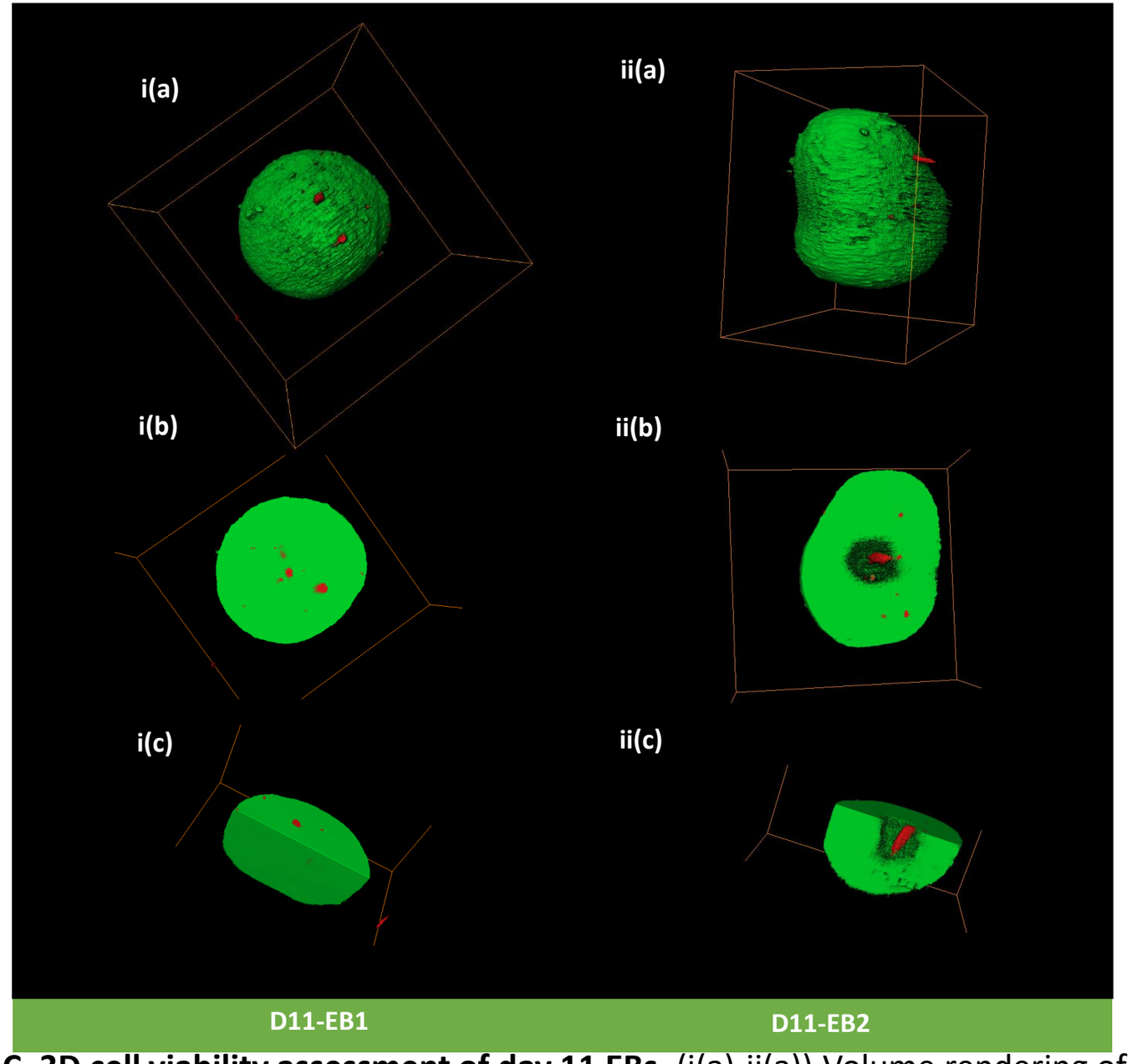
Supplementary figure 3



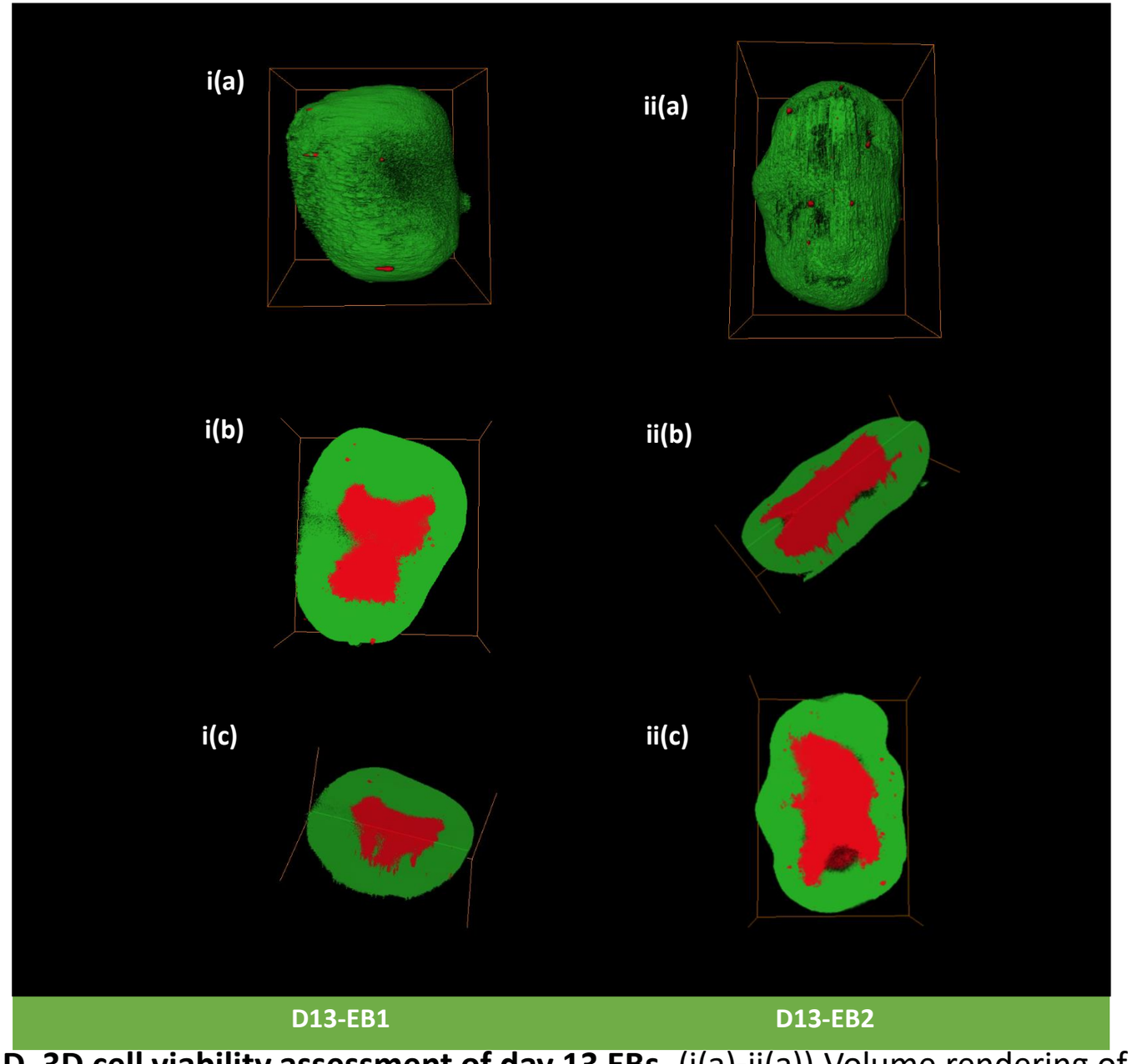
Supplementary Figure 3A. 3D cell viability assessment of day 7 EBs. (i(a)-ii(a)) Volume rendering of EBs. (i(b)-ii(b)) Cross-sections taken from the middle of the EBs.(i(c)-ii(c)) Further cross-sectional view of i(b)-ii(b). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.



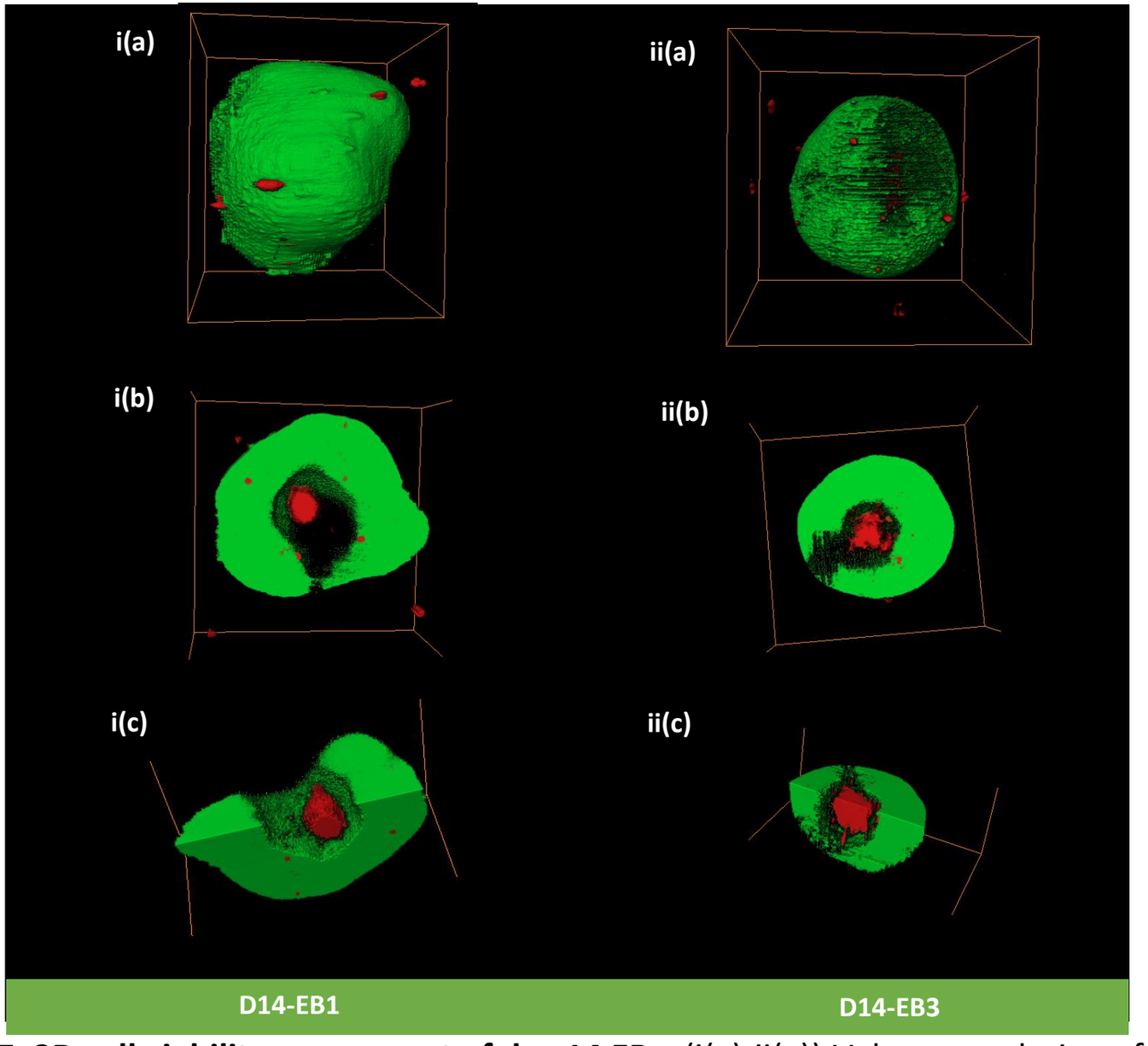
Supplementary Figure 3B. 3D cell viability assessment of day 10 EBs. (i(a)-ii(a)) Volume rendering of EBs. (i(b)-ii(b)) Cross-sections taken from the middle of the EBs. (i(c)-ii(c)) Further cross-sectional view of i(b)-ii(b). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.



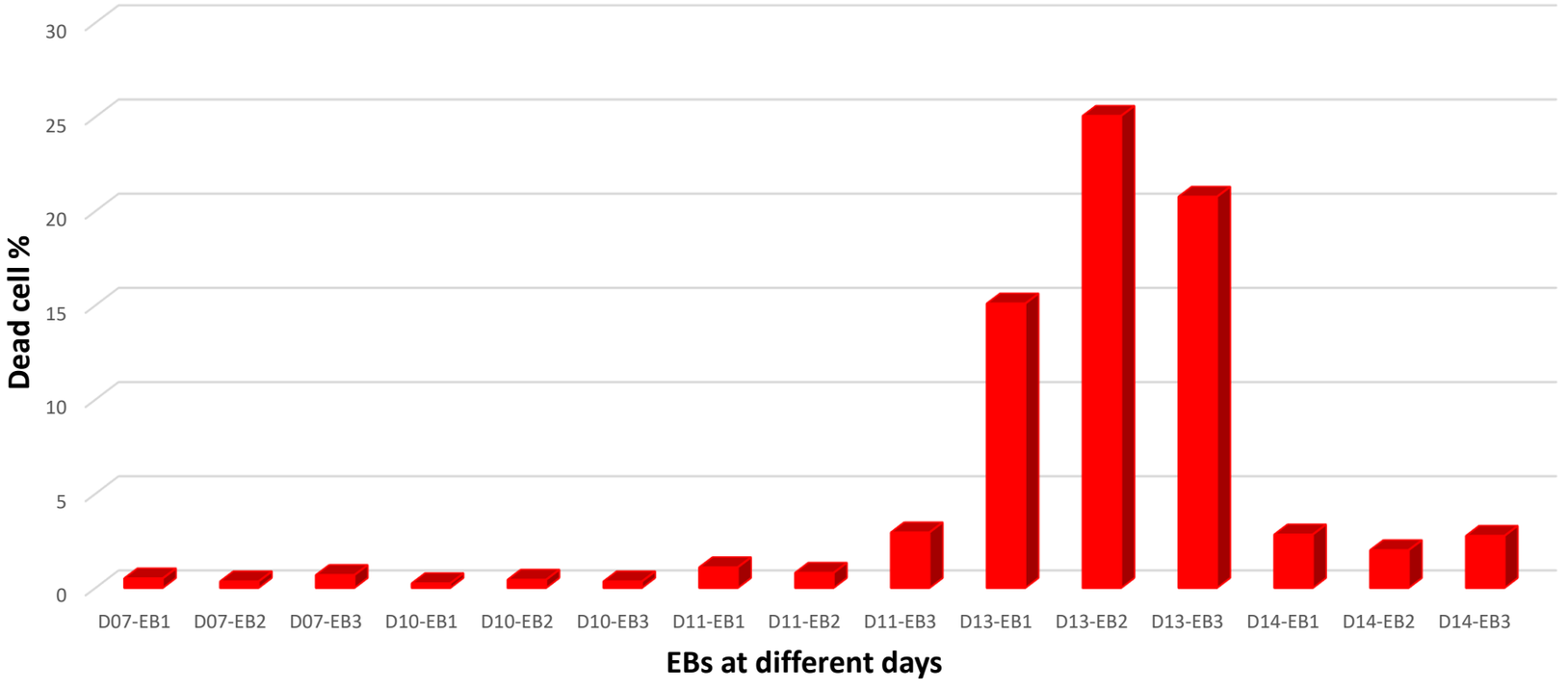
Supplementary Figure 3C. 3D cell viability assessment of day 11 EBs. (i(a)-ii(a)) Volume rendering of EBs. (i(b)-ii(b)) Cross-sections taken from the middle of the EBs. (i(c)-ii(c)) Further cross-sectional view of i(b)-ii(b). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.



Supplementary Figure 3D. 3D cell viability assessment of day 13 EBs. (i(a)-ii(a)) Volume rendering of EBs. (i(b)-ii(b)) Cross-sections taken from the middle of the EBs. (i(c)-ii(c)) Further cross-sectional view of i(b)-ii(b). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.

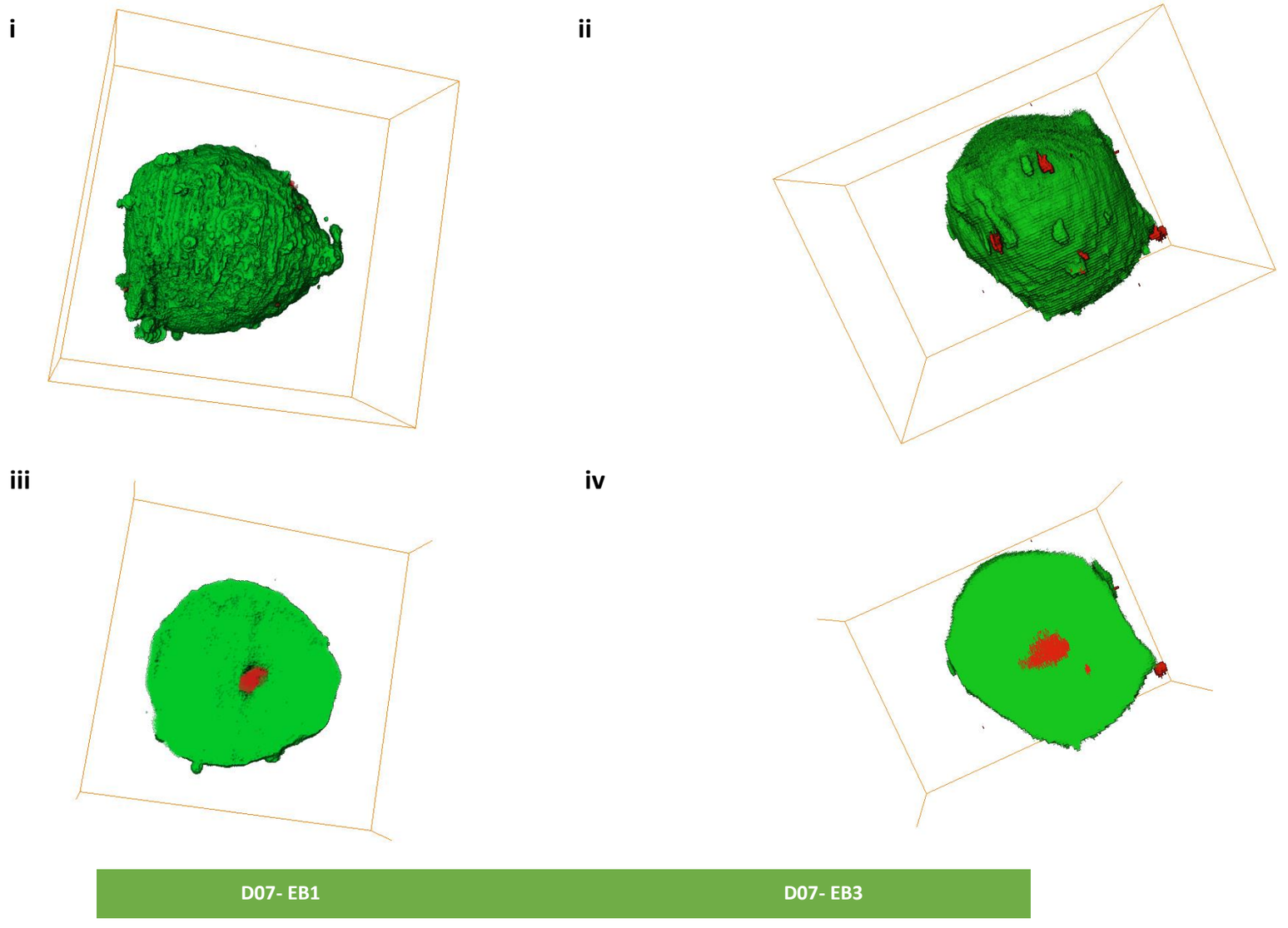


Supplementary Figure 3E. 3D cell viability assessment of day 14 EBs. (i(a)-ii(a)) Volume rendering of EBs. (i(b)-ii(b)) Cross-sections taken from the middle of the EBs. (i(c)-ii(c)) Further cross-sectional view of i(b)-ii(b). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.

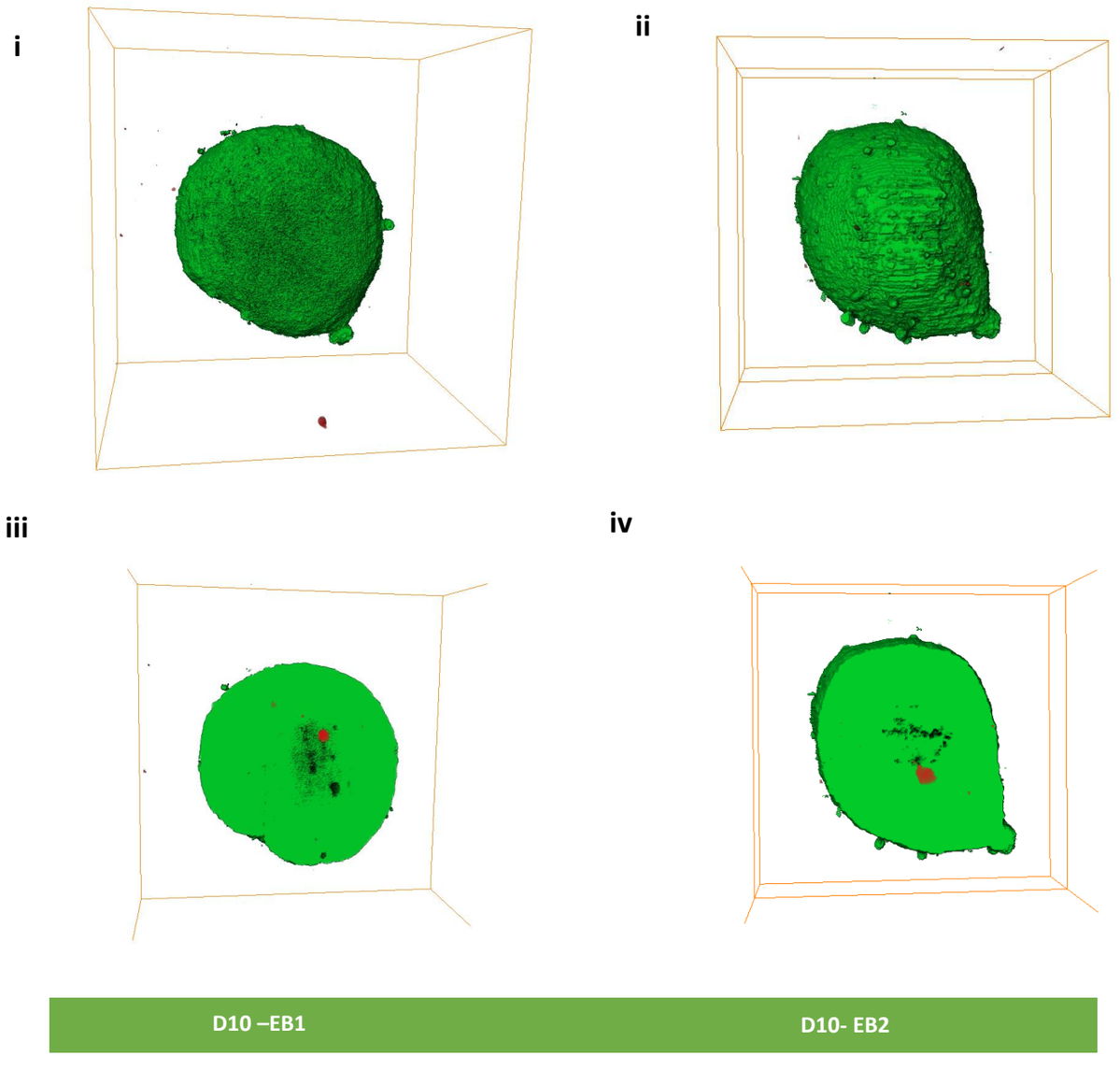


Supplementary Figure 3F. Dead cell percentage of EBs at different days.

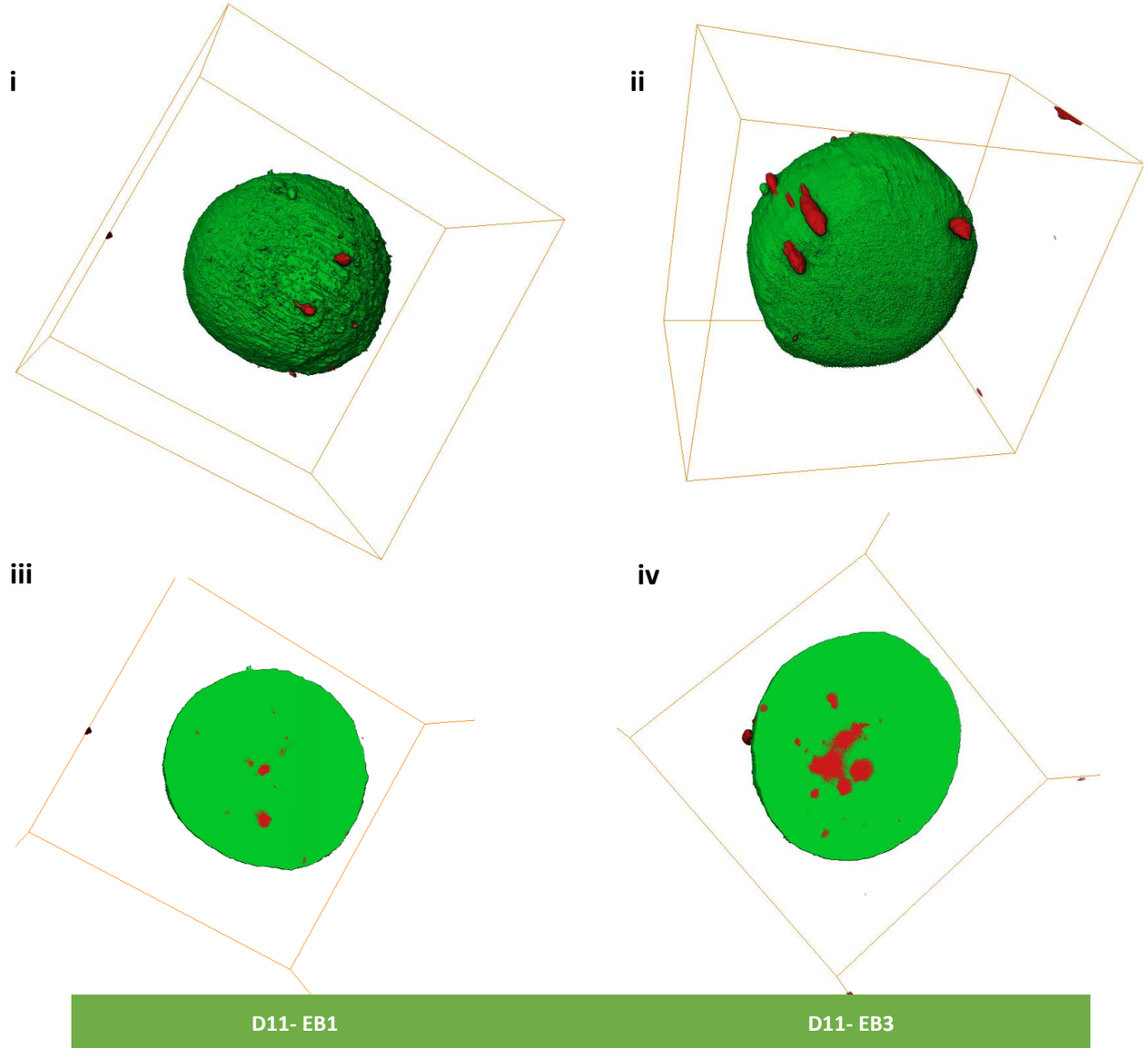
Supplementary figure 4



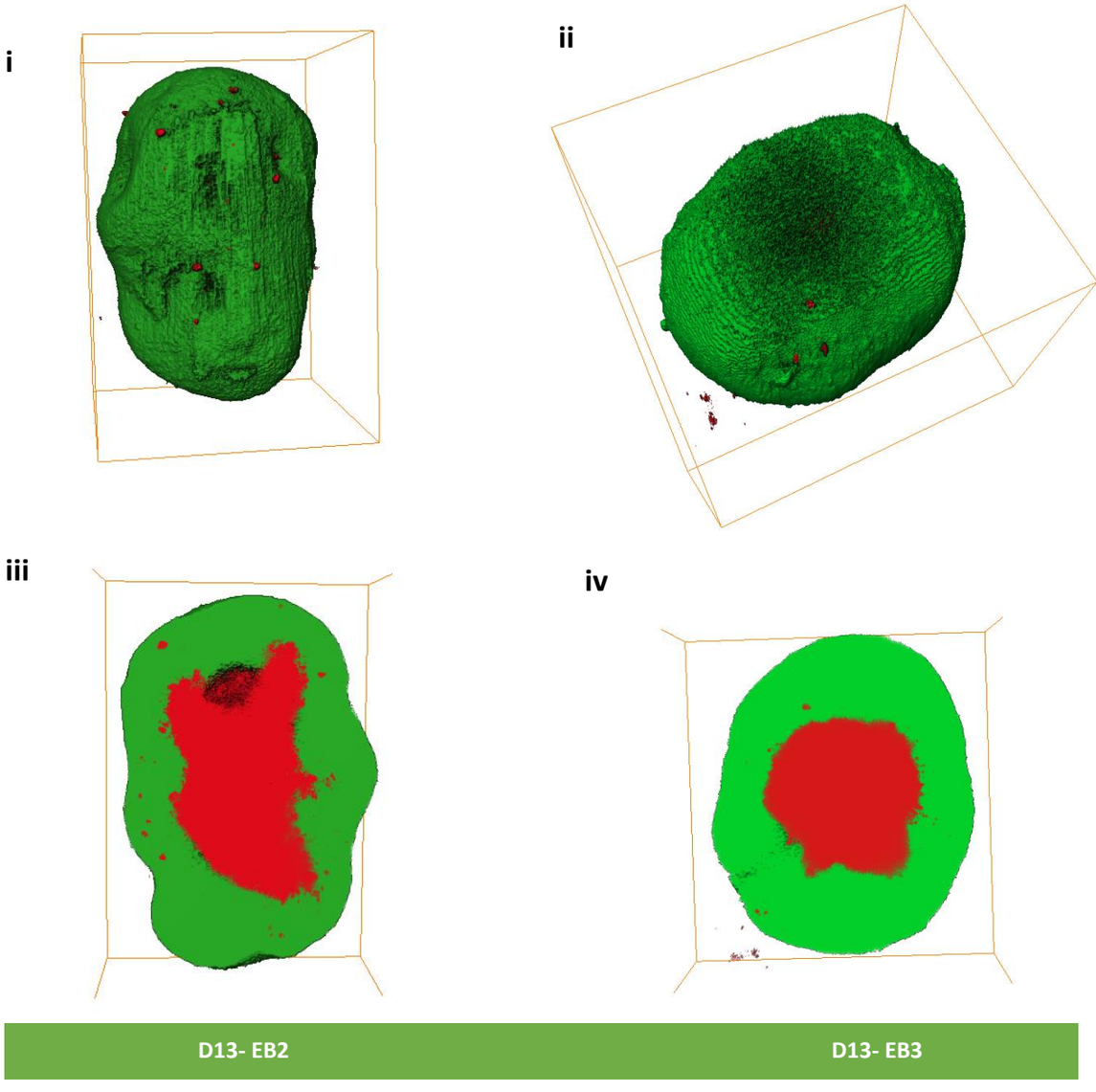
Supplementary Figure 4A. Morphological analysis of day 7 EBs. (i-ii) Volume rendering of EBs. (iii-iv) Cross-sections taken from middle of the EBs (i-ii). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.



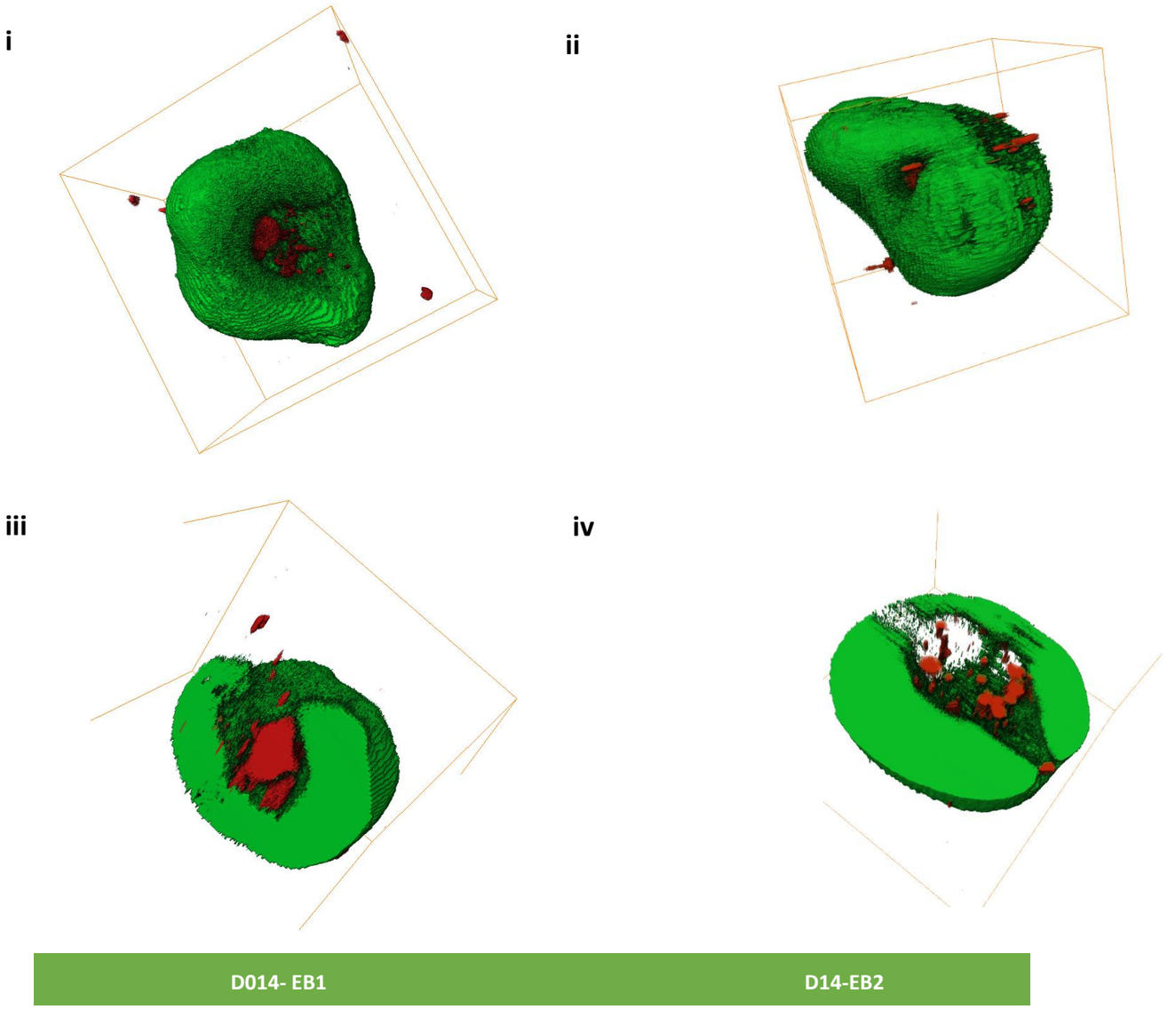
Supplementary Figure 4B. Morphological analysis of day 10 EBs. (i-ii) Volume rendering of EBs. (iii-iv) Cross-sections taken from middle of the EBs (i-ii). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.



Supplementary Figure 4C. Morphological analysis of day 11 EBs. (i-ii) Volume rendering of EBs. (iii-iv) Cross-sections taken from middle of the EBs (i-ii). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.

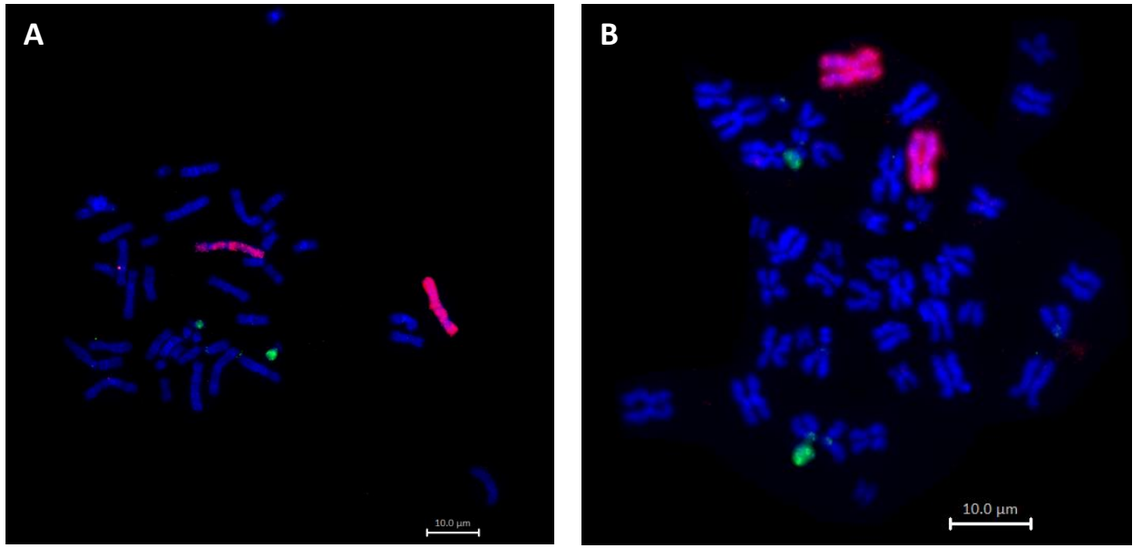


Supplementary Figure 4D. Morphological analysis of day 13 EBs. (i-ii) Volume rendering of EBs. (iii-iv) Cross-sections taken from middle of the EBs (i-ii). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.



Supplementary Figure 4E. Morphological analysis of day 14 EBs. (i-ii) Volume rendering of EBs. (iii-iv) Cross-sections taken from middle of the EBs (i-ii). Green shows live and red shows dead cells. Bounding box size for each EB is given in Supplementary Table 1.

Supplementary figure 5



Supplementary Figure 5. Two-color FISH on iPSC metaphase chromosomes. Direct labelled chromosome-specific "paints" for chromosome 2 (*red*) and 22 (*green*) on A) ipsc spread from a 2D culture and B) 3D EBs at day 8.

Supplementary Table 1

Embryoid Bodies at different days	Bounding box size (µm)	Total slices in XY	Diameter (µm)	Average Diameter (EB1-4) (µm)	Live cell volume ($µm^3$)	Dead cell volume ($µm^3$)	Inside Dead cell volume ($µm^3$)	Outside dead cell volume ($µm^3$)	Total volume (live + dead)($µm^3$)	Average volume (EB 1-3) (µm3)	Live volume %	Dead volume %	Dead cell average %	Inside dead cells %	Outside dead cells %
D07-EB1	359x364x231	77	181.77	184.2166667	3822112.848	18951.20361	18951.20361	0	3841064.051	3583371.523	99.5	0.5	0.50	0.5	0
D07-EB2	359x364x192	64	180.87		1973140.668	6617.781669	4575.787548	2041.994121	1979758.449		99.66	0.34		0.235	0.105
D07-EB3	360x365x249	83	190.01		4895872.141	33419.93564	29404.14868	4015.786966	4929292.076		99.32	0.68		0.60	0.08
D10-EB1	475x467x351	117	276.6	270.4166667	9423933.172	22893.32997	21798.8899	1094.44007	9446826.501	11334193.78	99.76	0.24	0.33	0.23	0.01
D10-EB2	476x467x282	94	254.67		10450121.77	44773.64965	44773.64965	0	10494895.41		99.57	0.43		0.43	0
D10-EB3	475x467x345	115	279.98		14013668.98	47190.4698	47190.4698	0	14060859.44		99.66	0.34		0.34	0
D11-EB1	475x467x366	122	274.09	276.82	14543273.25	159610.8052	144822.2952	14788.51007	14702884.05	14665360.41	98.91	1.09	1.61	0.99	0.1
D11-EB2	475x467x345	115	269.65		13736724.6	109700.3132	104898.1802	4802.133041	13846424.91		99.20	0.8		0.77	0.03
D11-EB3	476x467x333	111	286.72		14992731.13	454041.1475	450955.8674	3085.280073	15446772.28		97.06	2.94		2.92	0.02
D13-EB1	485x523x489	163	489.89	422.5	38057758.67	6762949.543	6762949.543	0	44820708.21	44229167.08	84.91	15.09	20.3	15.09	0
D13-EB2	688x472x480	154	399.76		39292301.23	13135385.23	13135385.23	0	52427686.46		74.95	25.05		25.05	0
D13-EB3	486x523x414	138	377.85		28081311.84	7357794.734	7357794.734	0	35439106.57		79.24	20.76		20.76	0
D14-EB1	515x523x441	147	441.94	395.3466667	23615142.76	686708.5602	671203.0767	15505.48347	24301851.32	21525354.42	97.17	2.83	2.53	2.77	0.064
D14-EB2	516x523x528	176	421.46		22175685.66	435108.9554	435108.9554	0	22610794.61		98.0	2.00		2.00	0
D14-EB3	515x523x387	129	322.64		17176419.02	486998.3299	461507.6214	25490.70844	17663417.35		97.24	2.76		2.616	0.144

Supplementary Table 1. Table summarizes the different parameters for EBs at different days of growth.

Supplementary Table 2

EBs at different days	Dead cells attached to the outer EB surface	Dead cells surrounding the EB	Dead cells inside EB	Dead cell (dense core or scattered around EB)
D07-EB1	Y	N	Y	DC and S
D07-EB2	Y	Y	Y	S
D07-EB3	Y	Y	Y	DC and S
D10-EB1	Y	Y	Y	S
D10-EB2	Y	N	Y	S
D10-EB3	Y	N	Y	S
D11-EB1	Y	Y	Y	S
D11-EB2	Y	Y	Y	S
D11-EB3	Y	Y	Y	DC and S
D13-EB1	Y	N	Y	DC and S
D13-EB2	Y	N	Y	DC and S
D13-EB3	Y	N	Y	DC and S
D14-EB1	Y	Y	Y	S
D14-EB2	Y	N	Y	S
D14-EB3	Y	Y	Y	DC and S

Supplementary Table 2. Table summarizes the visual cell viability assessment of EBs at different days of growth.
N = None; Y= Yes; S= Scattered; DC= Dense core

Supplementary Table 3

EBs at different days	Shape of EB	Inner EB cavities	Outer EB cavities
D07-EB1	Spherical	1 (surrounding dead cell core)	0
D07-EB2	Ellipsoidal	0	0
D07-EB3	Spherical	0	0
D10-EB1	Spherical	1 central cavity	0
D10-EB2	Spherical	1	0
D10-EB3	Spherical	1	0
D11-EB1	Spherical	0	0
D11-EB2	Ellipsoidal	1 central cavity	0
D11-EB3	Spherical	0	0
D13-EB1	Ellipsoidal	0	1 starting to appear
D13-EB2	Ellipsoidal	2 (2 surrounding dead cell core)	3 (connected to each other)
D13-EB3	Ellipsoidal	1 (appearing from one end of the dead cell core)	1 starting to appear
D14-EB1	Irregular	1 central cavity	1 (connected to the inner cavity)
D14-EB2	Ellipsoidal	1central cavity	4 (connected to the inner cavity)
D14-EB3	Spherical	1 central cavity	1 (Connected to the inner cavity)

Supplementary Table 3. Table summarizes the visual morphological assessment of EBs at different days of growth.

Supplementary Table 4

Chromosome spread number	2D iPSC	3D iPSC EB, day 8	3D iPSC EB, day 10	3D iPSC EB, day 11	3D iPSC EB, day 14
1	46,XY	46,XY	46,XY	46,XY	46, XY
2	46,XY	46,XY	46,XY	45,XY,-10	43XY,-19,-20,-21
3	46,XY	46,XY	46,XY	46,XY	45XY,-7
4	46,XY	46,XY	46,XY	46,XY	46, XY
5	46,XY	46,XY	46,XY	45,XY,-17	46, XY
6	46,XY	46,XY	46,XY	46,XY	46, XY
7	46,XY	46,XY	46,XY	46,XY	44X,-21 -Y
8	46,XY	46,XY	45,XY, -9	46,XY	44XY,-17,-22
9	46,XY	46,XY			46, XY
10	46,XY	46,XY			46, XY
11	46,XY	46,XY			46, XY
12	46,XY	46,XY			46, XY
13	46,XY	46,XY			46, XY
14	46,XY	46,XY			
15	46,XY	46,XY			

Supplementary Table 4. Table summarizes the karyotypic findings from the iPSCs (2D) and derived EBs (3D) at different days of growth using M-FISH.

Supplementary Table 5

Chromosome spread number	2D iPSC	3D iPSC EB, day 8	3D iPSC EB, day 10	3D iPSC EB, day 11	3D iPSC EB, day 14
1	46,XY	46,XY	46,XY	46,XY	46,XY
2	46,XY	46,XY	46,XY	46,XY	46,XY
3	46,XY	46,XY	46,XY	46,XY	46,XY
4	46,XY	46,XY	46,XY	46,XY	46,XY
5	46,XY	46,XY	46,XY	46,XY	46,XY
6	46,XY	46,XY	46,XY	46,XY	45,XY
7	46,XY	46,XY	46,XY	46,XY	46,XY
8	46,XY	46,XY	46,XY	46,XY	46,XY
9	46,XY	46,XY	46,XY	46,XY	43,XY
10	46,XY	46,XY	46,XY	46,XY	44,XY
11	46,XY	46,XY	46,XY	46,XY	46,XY
12	46,XY	46,XY	46,XY	46,XY	44,XY
13	46,XY	46,XY	46,XY	46,XY	46,XY
14	46,XY	46,XY	46,XY	46,XY	46,XY
15	46,XY	46,XY	46,XY	46,XY	46,XY
16	46,XY	46,XY	46,XY	46,XY	46,XY
17	46,XY	46,XY	46,XY	46,XY	46,XY
18	46,XY	46,XY	46,XY	45,XY	46,XY
19	46,XY	46,XY	46,XY	46,XY	46,XY
20	46,XY	46,XY	46,XY	44,XY	46,XY
21	46,XY	46,XY	46,XY	46,XY	46,XY
22	46,XY	46,XY	46,XY	46,XY	46,XY
23	46,XY	46,XY	46,XY	45,XY	46,XY
14	46,XY	46,XY	46,XY	46,XY	46,XY
15	46,XY	46,XY	46,XY	45,XY	46,XY
16	46,XY	46,XY	46,XY	46,XY	46,XY
27	46,XY	46,XY	46,XY	45,XY	46,XY
28	46,XY	46,XY	46,XY	46,XY	44,XY
29	46,XY	46,XY	46,XY	46,XY	46,XY
30	46,XY	46,XY	46,XY	46,XY	46,XY

Supplementary Table 5. Table summarizes the chromosomal count of iPSCs (2D) and derived EBs (3D) at different days of growth done on DAPI stained chromosome spreads.