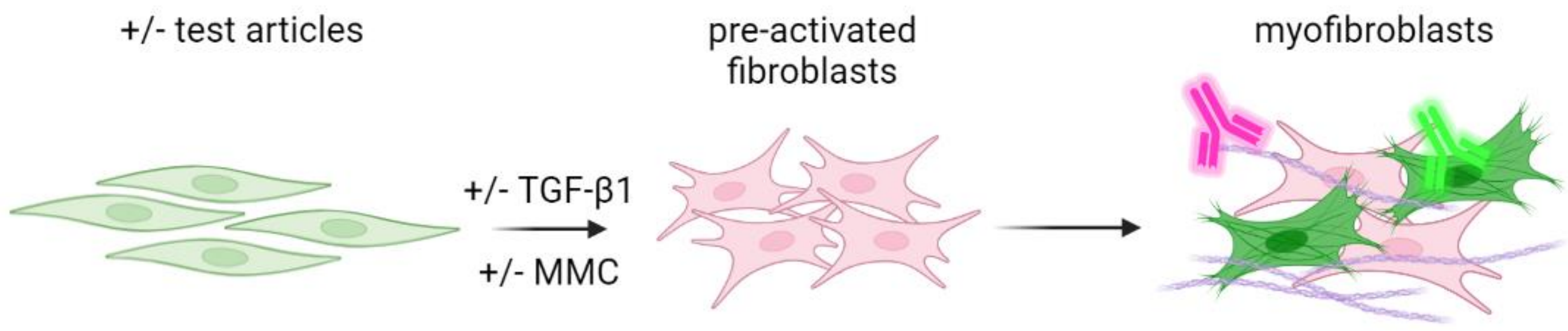
Overcoming technical difficulties of *in-vitro* assay-development.

Fiona Leslie, Chloe Whiting, Megan Webster

Newcells Biotech Limited, The Biosphere, Draymans Way, Newcastle Helix, Newcastle upon Tyne. UK NE4 5BX

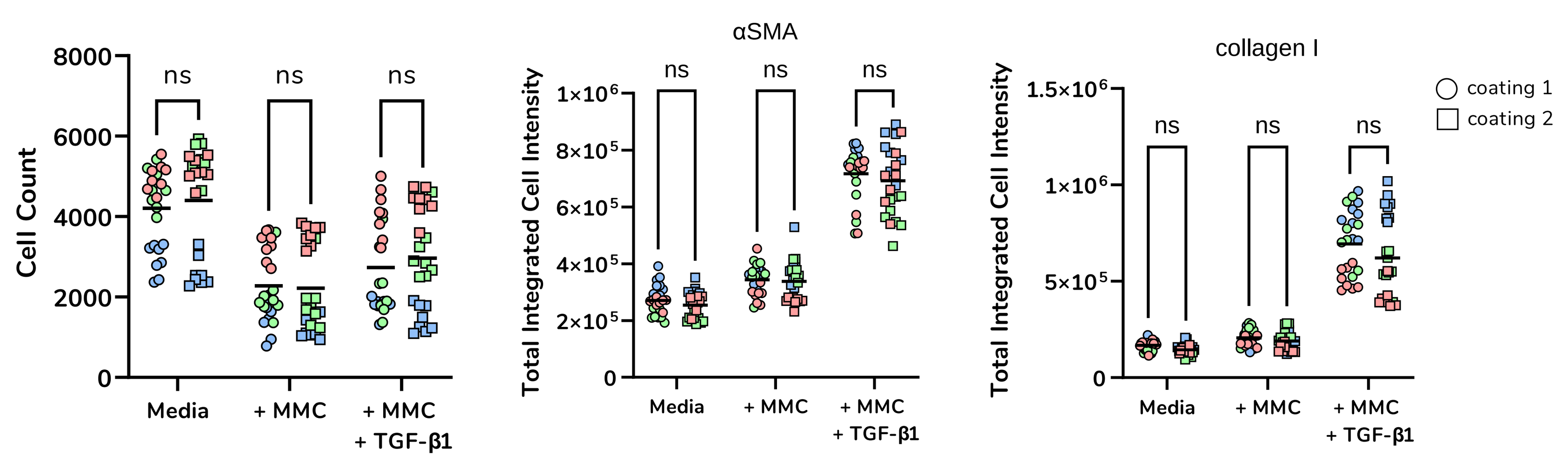
Modelling Fibroblast-to-Myofibroblast Transition (FMT)

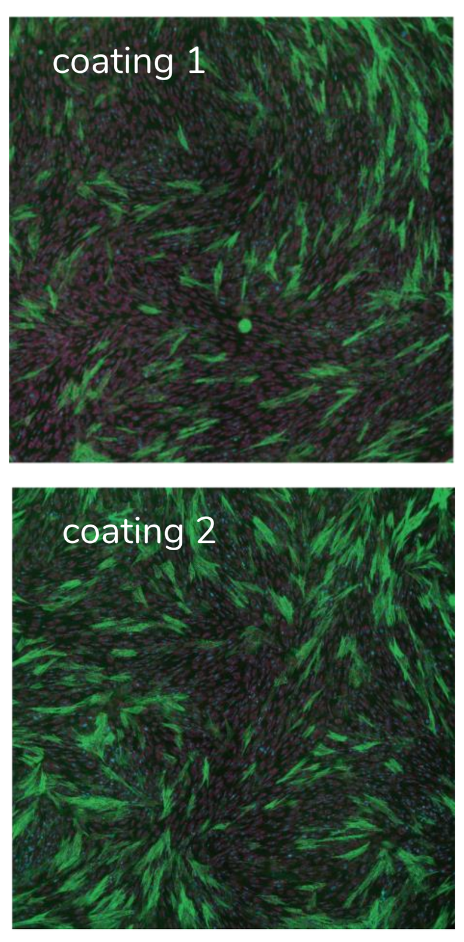
- TGF-β1 mediated activation of fibroblasts, their transition to α-SMA expressing myofibroblasts, and associated increase in the expression and deposition of extracellular matrix proteins represents one of the underlying pathologic mechanisms of fibrosis.
- Modelling this process *in-vitro* allows the testing of potential anti-fibrotic therapeutics for their ability to reduce or reverse FMT.

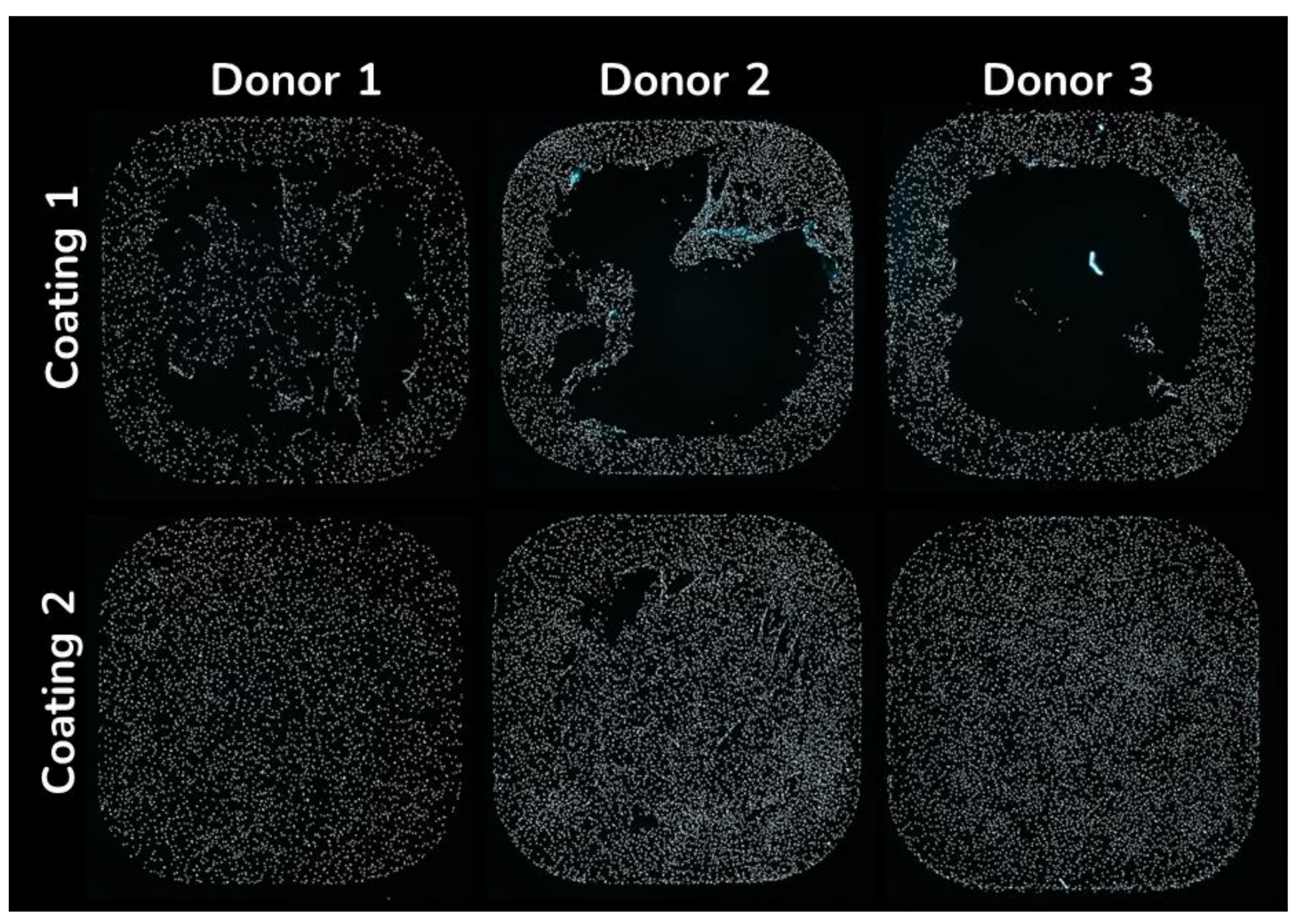


- The development and validation of robust assay systems requires significant time and effort. Our team worked to overcome multiple technical challenges to optimise our high-throughput, high-content imaging FMT-assay. Here we highlight some key difficulties and resolved outcomes.

Optimization of Plate Coatings

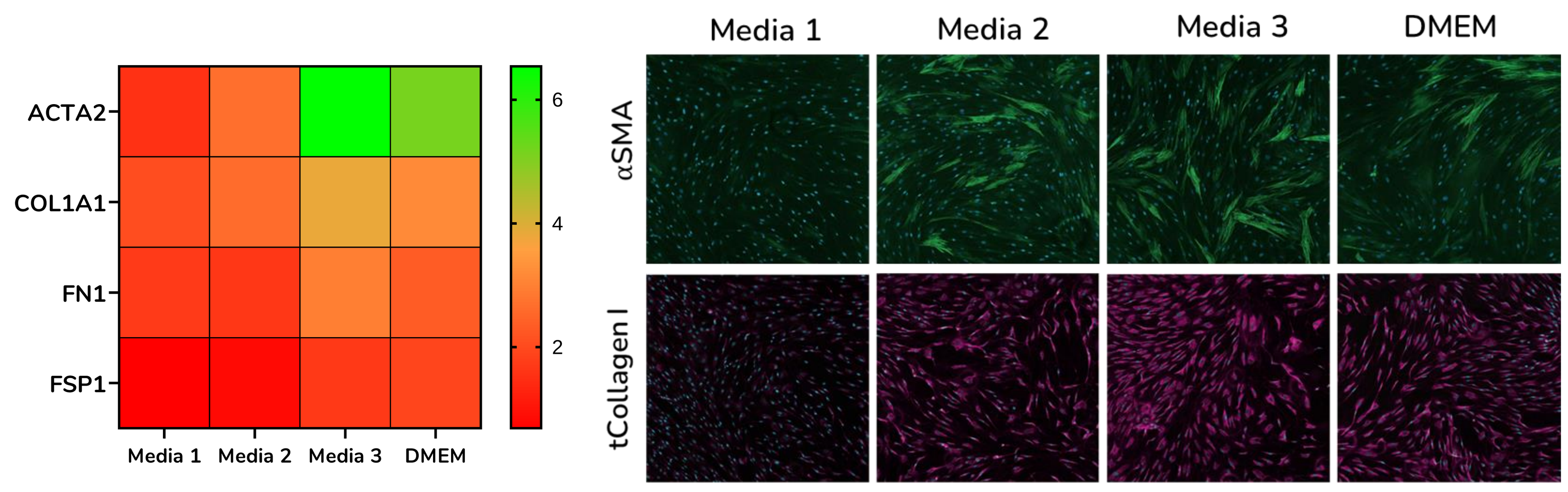


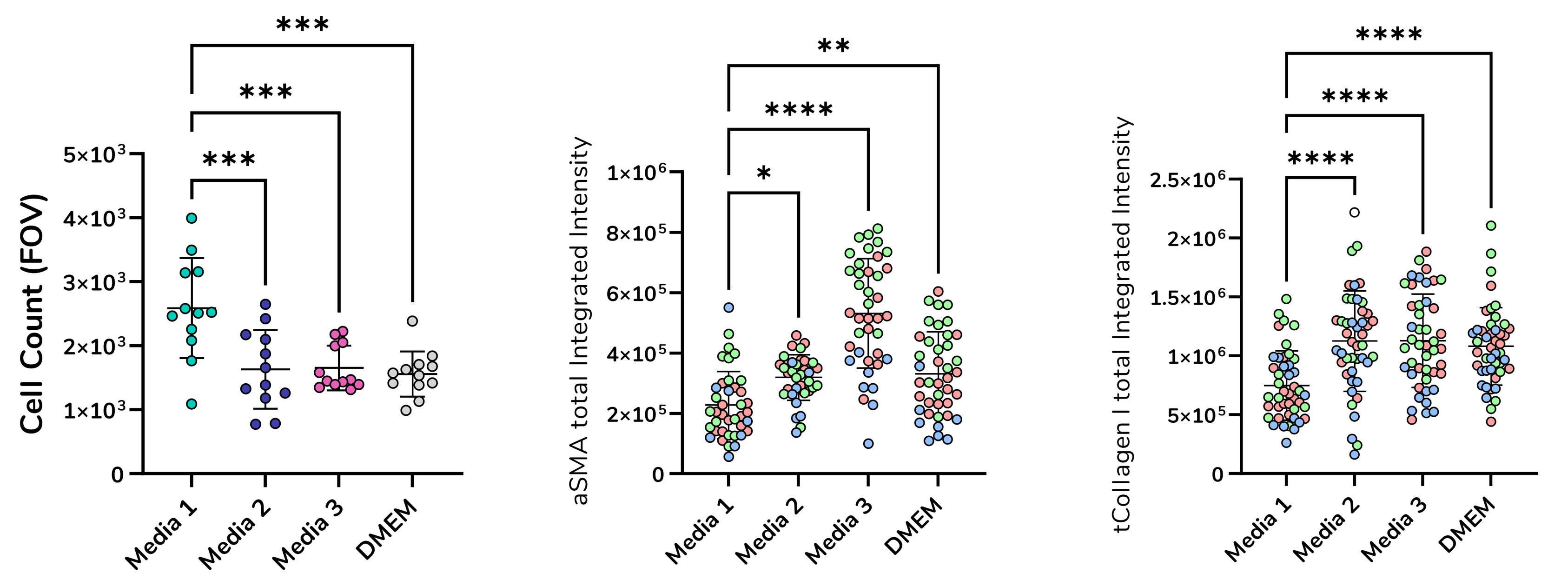




To ensure consistent adherence of cells to culture wells throughout the FMT-assay process, including mechanical washing, we optimized cell seeding densities and culture media to control for cell proliferation (data not shown). After observing significant cell detachment due to mechanical washing, we compared plate coatings to promote fibroblast attachment and reduce assay variation. Further work was performed to optimized immunocytochemical detection of both αSMA and collagen I.

Optimization of Culture Media





Ensuring a consistent, substantial, assay window is vital to establishing a reproducible in-vitro assay for compound screening. To promote the activation of primary fibroblasts following stimulation with TGF-β1 we worked to optimize assay culture media. As shown above, media 3 promoted the expression of both αSMA and collagen1 at the gene and protein level. Together with our refined segmental analysis masks we can accurately detect changes in the expression and deposition of αSMA and collagen I in response to treatment with potential therapeutic compounds.

Optimization of Plate Coatings

Collagen I

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B	4633	360102	376803	357530	297886	317401	325921	359880	305262	364636	310490	379652	174563	330468	229237	384371	281691	337497	316636	364475	359855	330675	367648	
C	16	272434	34[illegible]510	283351	284245	351071	351950	425918	489174	635092	767552	865093	1440343	1130072	1433263	1051188	1185452	1249012	1143987	253380	330708	353[illegible]73	338874	
D	8385	500832	421[illegible]03	486519	297952	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	271979	327736	338[illegible]99	312972	
E	0	468454	459[illegible]57	436511	245674	381946	338427	462147	370836	751738	784975	1026314	1098517	1318065	1232523	1083192	1151910	1192048	1213294	276215	408110	338[illegible]48	357307	
F	21	853607	70[illegible]054	941663	282607	366240	321529	382983	363348	507867	551417	1089911	1472189	1630538	1159491	1119638	1082603	1109849	1092655	263415	543768	658[illegible]45	563684	
G	0	1108002	622[illegible]40	843896	258357	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	248422	560495	598[illegible]78	512076	
H	0	1226594	825[illegible]86	1010101	343001	321771	344687	331509	350282	515995	559004	965888	1277114	1055511	1064798	1057162	1098613	1024389	1065289	294162	789513	867[illegible]12	957399	
I	23942	1506952	938[illegible]95	914375	264243	379832	359031	358451	397561	693818	791872	636833	856870	915768	950782	893949	950396	1071572	1019137	222072	496046	911[illegible]49	830994	
J	191409	1179077	116[illegible]336	1169024	256740	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	229467	989988	1017[illegible]45	910553	
K	222188	1280300	103[illegible]950	1200143	273853	365778	350147	416459	387146	770361	693754	804532	797821	1012703	971311	1059127	951964	883631	964787	228044	1036274	102[illegible]033	907661	
L	177907	1246709	106[illegible]392	1089907	308139	321324	343088	374730	303012	577052	519399	891798	1123668	1270865	1319397	1111476	1119724	997700	1018140	283169	1140993	109[illegible]373	1141343	
M	0	1181769	936[illegible]97	1094163	264428	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	188335	991631	962[illegible]85	1020549	
N	0	1309429	932[illegible]64	852190	299820	240846	259964	244450	341527	534334	526545	783785	1317273	1328636	1187583	1235584	971261	1025072	852505	289026	986804	848[illegible]86	1011153	
O	0	1078883	93[illegible]675	1006435	328528	231208	257474	223995	315550	330499	334706	269599	258442	346454	319082	365100	217365	254486	274515	220232	1173662	101[illegible]01	1233786	
P																								

αSMA

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B	2064	11353	9[illegible]21	7165	20371	20269	13838	9878	18311	21862	13685	22088	44679	25252	57044	14652	5208	12211	9976	13297	17425	12063	15028	
C	1153	8782	13[illegible]68	25716	19882	14046	13397	9193	13355	43132	56915	133032	59892	491431	152340	710302	562485	578133	622502	8055	14911	19[illegible]15	8089	
D	271	8690	30[illegible]92	8940	18978	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	22361	18408	23[illegible]37	19618	
E	15575	33597	14[illegible]02	7506	52396	5566	13207	19974	15283	34728	24258	98170	80445	348444	202641	444765	664016	591296	603184	10600	11210	19[illegible]41	23614	
F	22135	58189	62[illegible]88	21153	14003	11643	19512	19229	18170	24377	18514	84562	55018	371784	237546	598096	597534	535448	604103	11119	17495	32[illegible]28	13711	
G	16601	35543	74[illegible]74	24186	32518	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11982	14530	16[illegible]46	57720	
H	8293	50744	10[illegible]727	47477	8714	9550	20506	7364	24029	24737	29233	59133	70919	308689	403383	454565	547393	416167	584612	12812	77873	40[illegible]25	44996	
I	2852	60169	52[illegible]92	63950	28155	16612	11916	14434	27482	41561	47228	172091	127149	407085	506347	584320	656136	620251	602065	31938	66584	25[illegible]61	68952	
J	980	358397	494[illegible]41	304646	24288	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	20624	424373	112004	240240	
K	1903	98819	40[illegible]204	493849	15536	21734	15578	15348	18554	57481	30882	142724	146274	396527	348814	665861	581931	528135	500484	17307	413443	13[illegible]764	191593	
L	3760	116603	60[illegible]540	577787	17315	5116	20567	7258	19919	21917	22086	126884	82987	479135	239554	676135	627486	567126	600368	18768	499991	65[illegible]07	168564	
M	678	266126	59[illegible]116	434374	26768	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	23909	390660	37[illegible]142	248372	
N	5293	109821	39[illegible]43	389674	23842	22749	16510	19264	21042	34662	24160	79104	75000	270841	168517	620401	574962	629898	479372	4557	509399	16[illegible]504	232898	
O	721	422566	634580	381880	10567	26299	14628	18025	21342	5640	20804	9140	15370	6194	26091	4036	7182	4556	7194	9896	482858	26[illegible]954	369576	
P																								

To ensure consistent data, independent of well position on the plate, αSMA and collagen I were quantified following the stimulation of primary fibroblasts with increasing TGF-β1 concentration across both the horizontal and vertical axis (arrows). The effects of two different stimulation methods were also tested (dashes). Applied conditional formatting shows increased expression of αSMA and collagen I, ranging green through red respectively.

Newcells Biotech's FMT-assay

- Newcells' FMT-assay utilises high-content imaging to determine the effects of potential therapeutics on fibroblast activation and collagen expression and deposition in high-throughput format.

Assay Features:

- 3 validated primary HLF donors
- High-throughput 384-well format
- 6-point dose response for up to 6 TAs per plate
- 6 technical replicates per condition
- Plus validated experimental assay controls

Available Assay Readouts:

- ✓ Cell Number
- ✓ Collagen I
- ✓ αSMA

Further details available online or contact us at
enquiries@newcellsbiotech.co.uk

Also available:
Small Airway Epithelial Cell (SAEC) model.