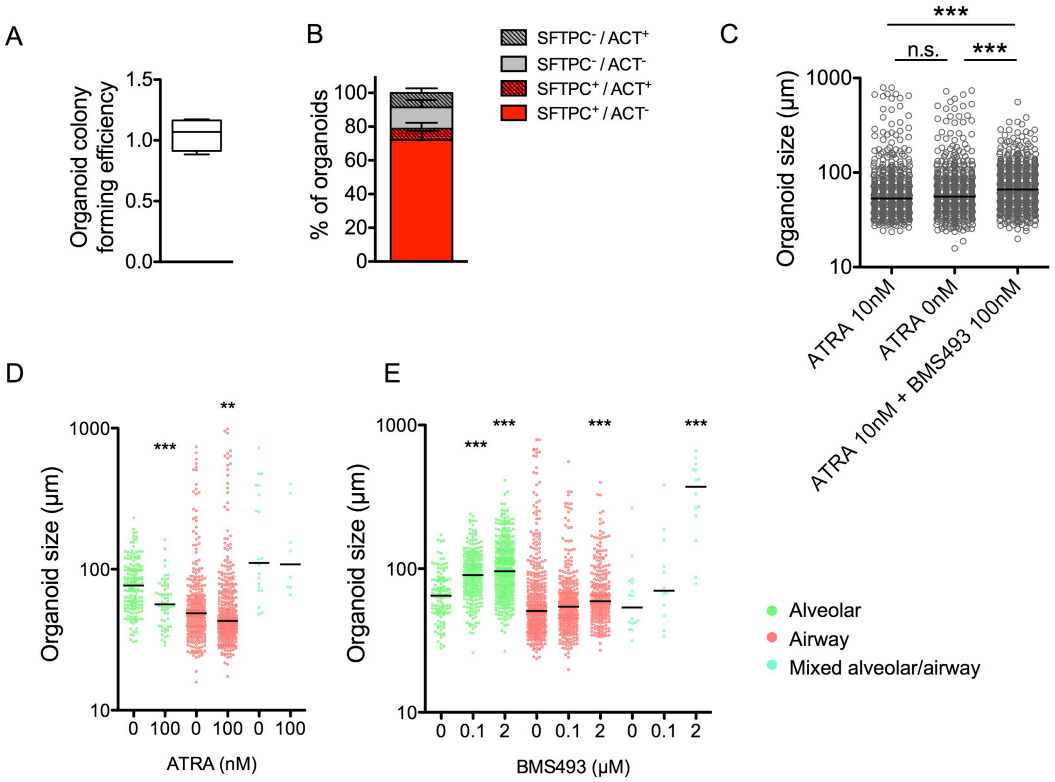
Supplemental Information

Fig. S1: Characterization of lung organoid model



A) Quantification of organoid number at day 7 incubated in normal organoid culture medium (identical data to Figure 1I, BMS493 [0nM]), expressed as a proportion of initial cells seeded. N=4 independent isolations. Whiskers represent min to max.

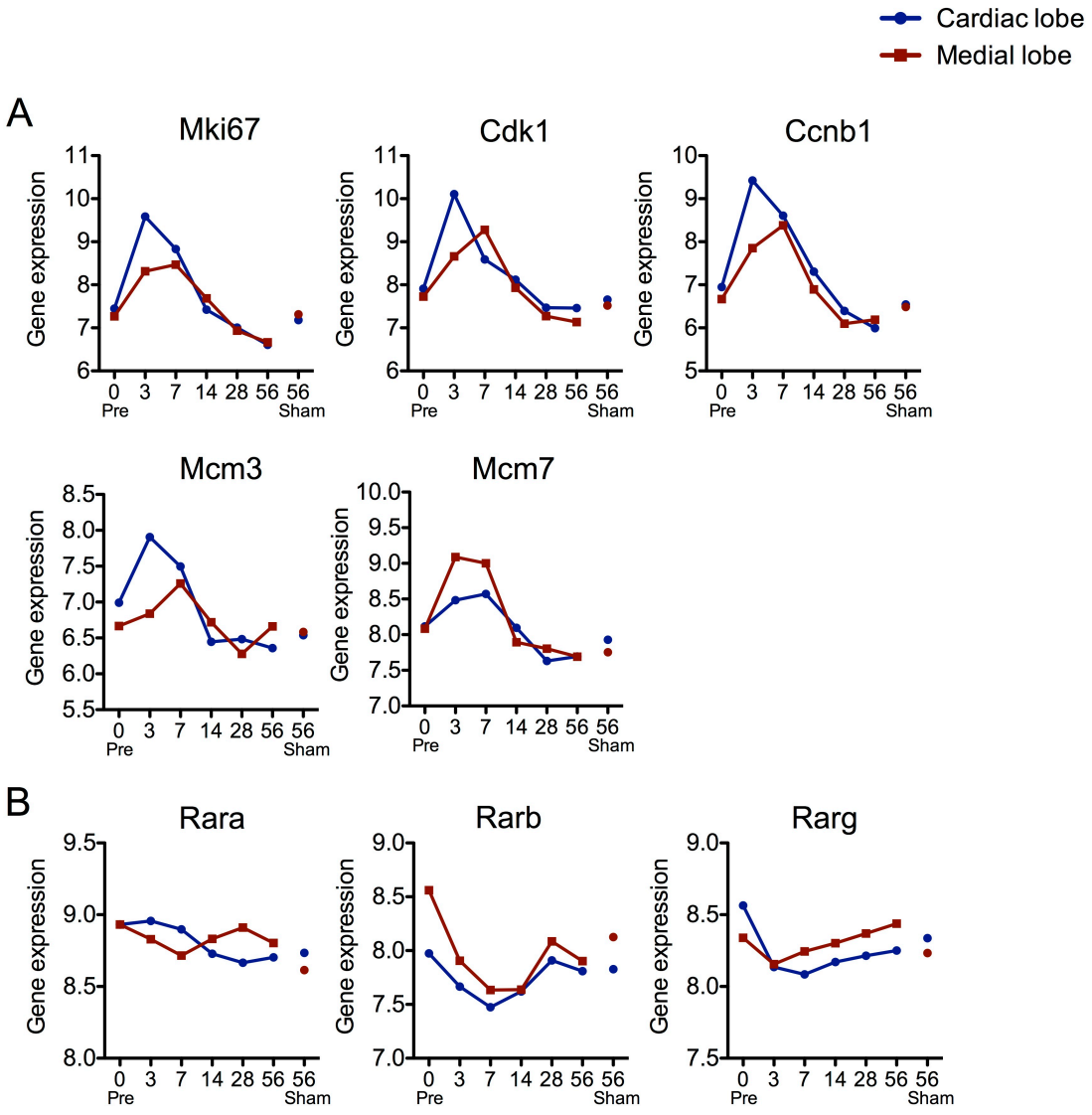
B) Proportion of organoids expressing SFTPC and/or ACT by immunofluorescence staining at day 14 incubated in normal organoid culture medium (identical data to Figure 2H, BMS493 [0nM]). N=3 independent isolations. Mean ± SEM.

C) Data from Figure 1E, separated according to organoid morphology. Effect of ATRA (0, 100nM) on diameter of alveolar (green), airway (red) and mixed alveolar/airway (blue) organoid subtypes. N=5 independent isolations. Horizontal line represents median. Mann Whitney test, **P<0.01, *** P<0.001, compared to corresponding 0nM control.

D) Data from Figure 1H, separated according to organoid morphology. Effect of BMS493 (0, 0.1, 2µM) on diameter of alveolar (green), airway (red) and mixed alveolar/airway (blue) organoid subtypes. N=5 independent isolations. Horizontal line represents median. Kruskall Wallis with Dunn's post test, *** P<0.001, compared to corresponding 0nM control.

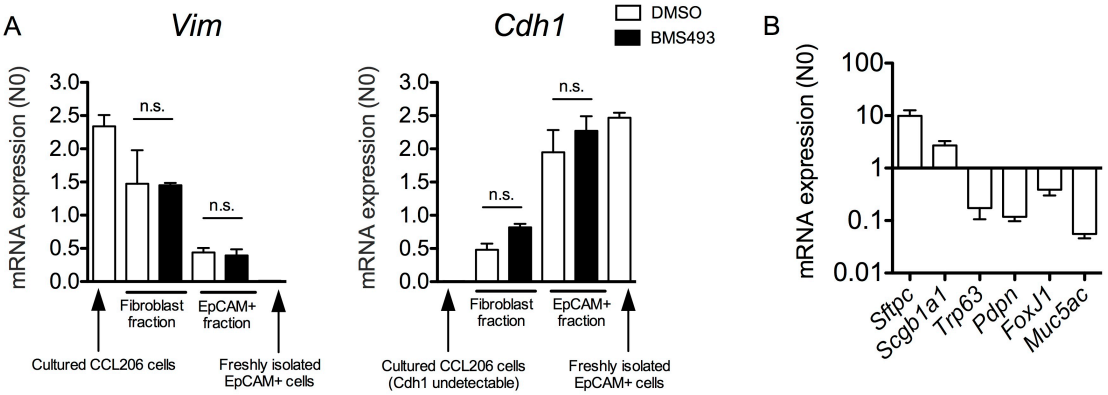
E) Effect of removal of ATRA from organoid culture medium (ATRA 0nM) or addition of BMS493 (100nM) compared to normal organoid culture medium (ATRA 10nM) on organoid size measured at day 14. Data from Figure 1E and 1H. N=5 independent isolations. Horizontal line represents median. Kruskall Wallis with Dunn's post test, n.s.=not significant, ***p<0.001.

Fig. S2: Analysis of published microarray datastet from Kho *et al*., 2013



Gene expression of A) proliferation markers and B) RA receptors in lung tissue samples from adult mice at various time points during post-pneumonectomy lung regeneration. Analysis of microarray data from (44) (GEO accession number GSE39817).

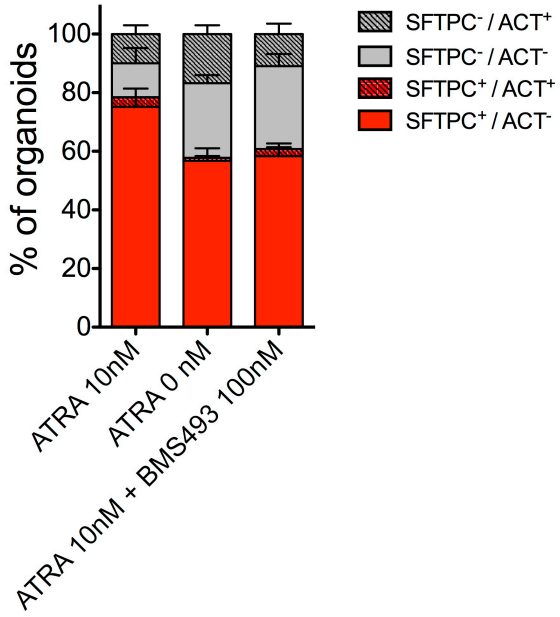
Fig. S3: Characterization of MACS-based re-sorting method



A) Re-sorting efficiency. Expression of the mesenchymal marker vimentin (*Vim,* left) and the epithelial marker E-cadherin (*Cdh1*, right) in re-sorted EpCAM$^+$ and fibroblast cell fractions from day 7 organoid cultures following treatment with DMSO (white bars) or BMS493 (2 µM, black bars). N=3 independent isolations. Unpaired t-test, n.s.=not significant compared to corresponding DMSO control.

B) RT-qPCR on EpCAM$^+$ cells re-sorted from organoid cultures at day 14, showing baseline expression of genes marking lung epithelial cell types. N=3-4 independent isolations. Mean ±SEM.

Fig. S4: BMS493 mirrors the effect of ATRA withdrawal on lung organoid differentiation



Effect of removal of ATRA from organoid culture medium (ATRA 0nM) or addition of BMS493 (100nM) compared to normal organoid culture medium (ATRA 10nM) on proportion of organoid expressing SFTPC and/or ACT by immunofluorescence at day 14. Data from Figure 2G and 2H. N=3 independent isolations. Mean ± SEM.

Table S1: Patient characteristics

Patient	Age	Sex	Smoking status	Packyears	FEV1, % pred	FVC, L	FEV1/FVC	GOLD stage
1	51	M	Ex	30	16	1.54	41	IV
2	64	M	Ex	30	21	2.27	37	IV
3	66	M	Ex	47	24	3.66	25	IV
4	74	M	Ex	N/A	62	3.90	50	II

Table S2: Antibodies used

Primary antibodies

Antibody	Host	Company	Dilution
Pro-Sftpc	Rabbit	Millipore (AB3786)	1:200
E-cadherin	Mouse	BD (610182)	1:200
Acetylated tubulin	Mouse	Sigma (T7451)	1:200
Ki67	Rat	Dako/Agilent (M7248)	1:200
β-tubulin IV	Mouse	Sigma (T7941)	1:100
YAP	Mouse	Santa Cruz (G-6, sc-376830)	1:100
SCGB3A2	Goat	R&D Systems (AF3465)	1:100

Secondary antibodies

Antibody	Host	Company	Dilution
Anti-mouse Alexa Fluor 488	Donkey	Thermo Fisher (10544773)	1:200
Anti-mouse Alexa Fluor 568	Donkey	Thermo Fisher (A10037)	1:200
Anti-goat Alexa Fluor 568	Donkey	Thermo Fisher (A11057)	1:200
Anti-rabbit Alexa Fluor 488	Donkey	Thermo Fisher (A21206)	1:200
Anti-rabbit Alexa Fluor 568	Goat	Thermo Fisher (A11008)	1:200
Anti-rat Alexa Fluor 647	Goat	Abcam (ab150159)	1:200

Table S3: Primers used

Primer		Sequence
Rpl13a	Forward	AGAAGCAGATCTTGAGGTTACGG
	Reverse	GTTCACACCAGGAGTCCGTT
B2M	Forward	TGCTATCCAGAAAACCCCTCAA
	Reverse	GGATTTCAATGTGAGGCGGG
Rarb	Forward	TAGAAAACGACGACCCAGCA
	Reverse	TGGGGTCAAGGGTTCATGTC
Cdk1	Forward	ACGGCTTGGATTTGCTCTCA
	Reverse	ACGATCTTCCCCTACGACCA
Ccnb1	Forward	GAGAAGGTGCCTGTGTGTGA
	Reverse	TCGGGCTTGGAGAGGGATTA

Ccnd1	Forward	GACCTTTGTGGCCCTCTGT
	Reverse	AGGCAGTCCGGGTCACA
Bcl2	Forward	TACGAGTGGGATGCTGGAGA
	Reverse	GAAGGAGAAGATGCCAGGGG
Bik	Forward	TCATGGAGTGCGTGGAAGG
	Reverse	CAGCGAGTCTGTGTATAGCAATC
Bax	Forward	AACTGGTGCTCAAGGCCC
	Reverse	TCCCGAAGTAGGAGAGGAGG
Trp63	Forward	GCATGGGAGCCAACATTCC
	Reverse	GCAGCCCAACCTTGCTAAGA
Sftpc	Forward	GGAGCACCGGAAACTCAGAA
	Reverse	GGAGCCGCTGGTAGTCATAC
Pdpn	Forward	TCACCCCAATAGAGATGGCTTG
	Reverse	GGGCAAGTTGGAAGCTCTCTT
Scgb1a1	Forward	ACCCTCCCACAAGAGACCA
	Reverse	GAGGAGCCGAGGAGACACA
Foxj1	Forward	ACTTCTGCTACTTCCGCCAT
	Reverse	CGAGGCACTTTGATGAAGCAC
Muc5ac	Forward	GAGATGGAGGATCTGGGTCA
	Reverse	GCAGAAGCAGGGAGTGGTAG
Vim	Forward	AGAGAGGAAGCCGAAAGCAC
	Reverse	TGGATCTCTTCATCGTGCAGT
Cdh1	Forward	ACGATCTTCCCCTACGACCA
	Reverse	GGGTTCCTCGTTCTCCACTC
Tead4	Forward	GGGAGATCCAGGCCAAACTC
	Reverse	TGCCAAAACCCTGAGATTGCT
Kif23	Forward	TGCTGACCCACCAGGAACTA
	Reverse	ATCTTCGTTTCCGACTACCAGTTG
Polh	Forward	ATGTGGGCGGATGATGCTAA
	Reverse	CTAGCTTCCCGGTACTTGGTG
Ccna2	Forward	ACCAAGAGAATGTCAACCCCG
	Reverse	AAGAGGAGCAACCCGTCG
Cdca5	Forward	TCATGCAGTAGAGGTCCCAGA
	Reverse	CTTGAGCGGAGGGTTGTTCT
Cdca8	Forward	GACAGACAGACCCTCCTCAAG
	Reverse	TCTGTGATGTCTCGATCAGCTTTT
Yap	Forward	AATGCAGTGTCTTCTCCTGGG
	Reverse	ATGGTGTCTCCTGTATCCATTTCA
Taz	Forward	GACCCTCATCTCTGGGGGAT
	Reverse	CCCATTTGAAGCGCAGGAAC
Fgf7	Forward	CTGCTCCACGCTAACTTCCA
	Reverse	GAGTTTACGCACCAGCACAC
Fgf10	Forward	GTCAGCGGGACCAAGAATGA
	Reverse	GTCGTTGTTAAACTCTTTTGAGCC

Timp3	Forward	GGTGGGAAAGAAGCTGGTGA
	Reverse	TTCATACACGCGCCCTGTC
Ctsh	Forward	TCATGGCTGCAAAGGAGGT
	Reverse	GAGCCACAGCCTCAACCAT
Tacstd2	Forward	CTGCTCCACGCTAACTTCCA
	Reverse	GAGTTTACGCACCAGCACAC
Tmod3	Forward	TGCGACCTTGCAGCTATTCT
	Reverse	TGGTTGGATTTGGTGGCTCA
Fgfr2b	Forward	TCCCCCTGCGGAGACA
	Reverse	TGCCCAGCGTCAGCTTAT