Supplementary Materials for:

TAZ is required for lung alveolar epithelial cell differentiation after injury

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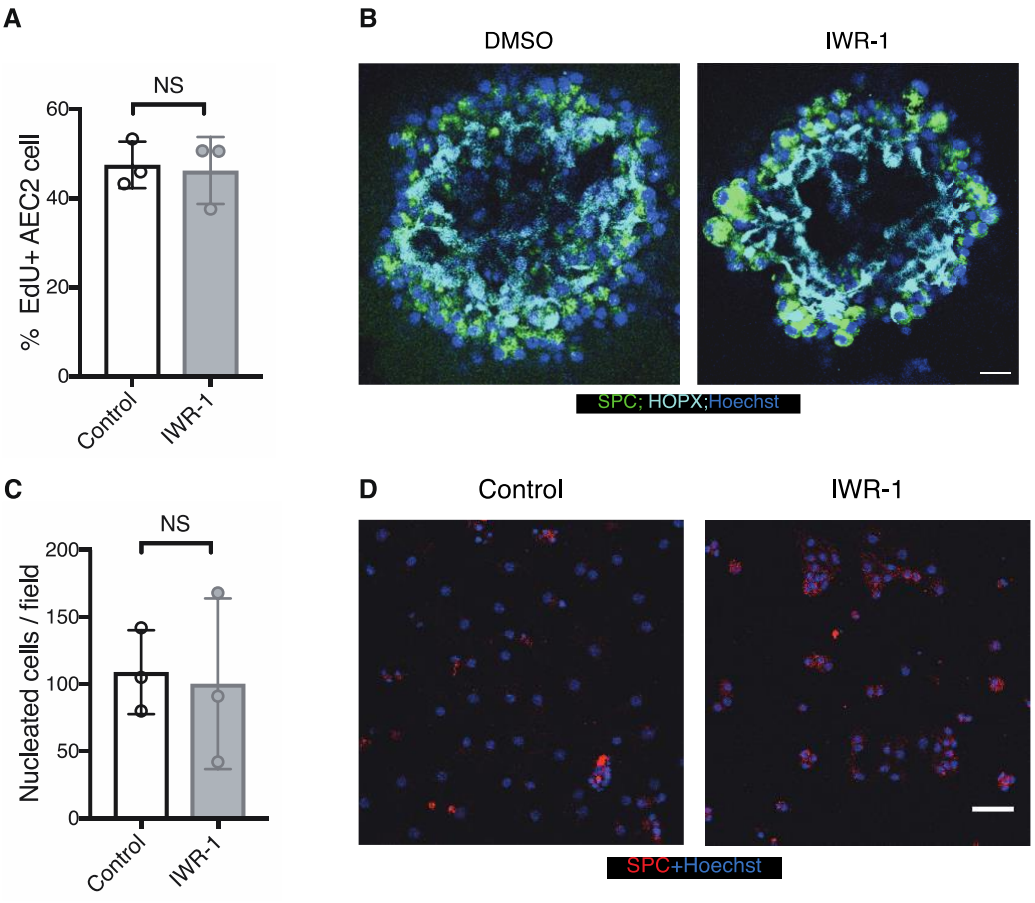
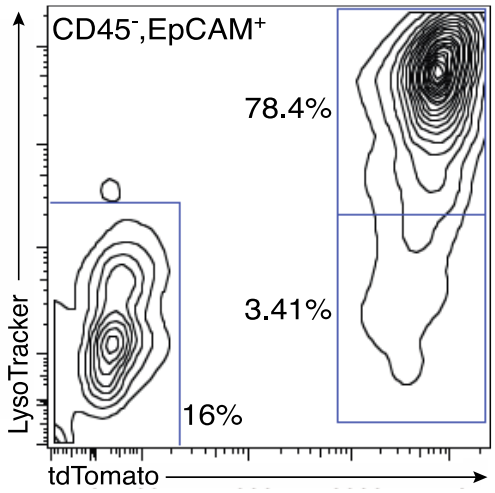
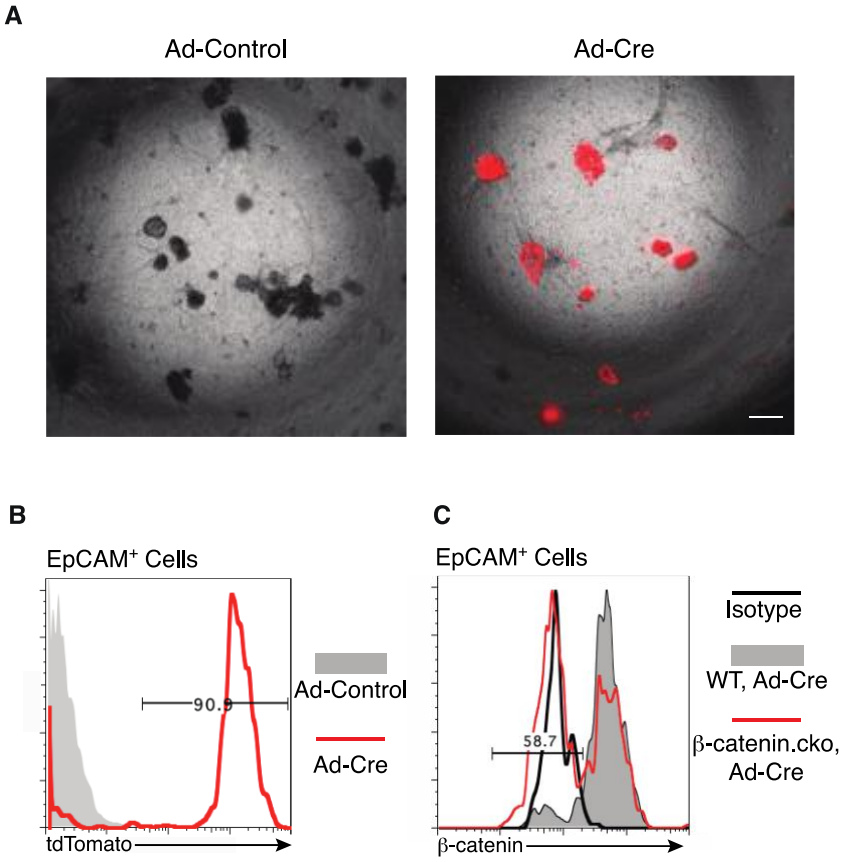


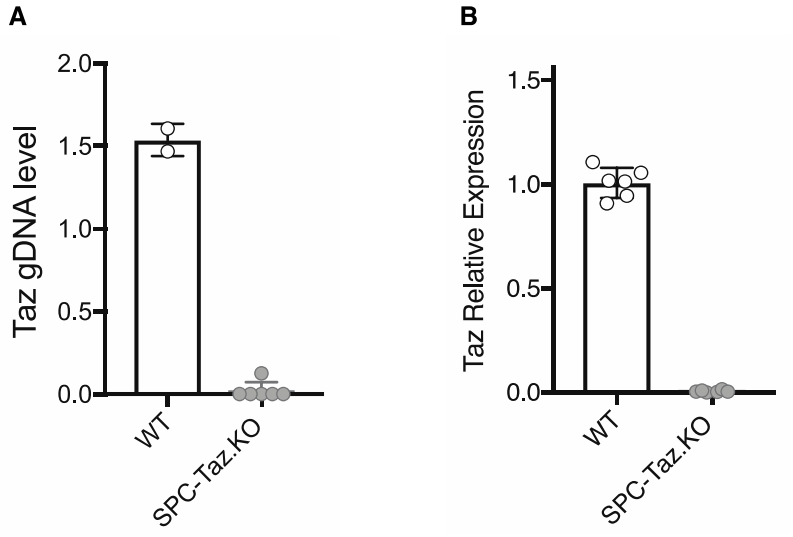
Supplemental Figure 1. **IWR-1 inhibits AEC2 to AEC1 differentiation in vitro. (A)** Proliferation rates of AEC2s are unaffected by IWR-1 treatment. AEC2s from SPC-WT-Tm were culture to form organoids as before. The cultures were pulsed with 10μM EdU for 2 hr after 6 days. Cells were collected afterwards and the percentages of EdU^+ among all $tdTomato^+$ AEC2s were determined by flow cytometry. **(B)** IWR-1 is not toxic to AEC1s that have already differentiated in alveolospheres. AEC2s were sorted from tamoxifen treated SPC-WT-Tm mice and cultured to form alveolospheres as before. After spheroids formed, cultures were treated overnight with either control (DMSO) or IWR-1 (10μM). Spheroids were then immunostained for SPC (AEC2) and HOPX (AEC1) and counterstained with Hoechst. TdTomato channel is omitted from these images for clarity. **(C and D)** AEC2s were sorted from C57B6 mice and directly plated on cell culture treated plates for 5 days with or without IWR-1. **(C)** Quantification shows the numbers of nucleated cells per image field. NS: not significant (unpaired two-tailed Student's *t*-test). **(D)** Cells were immunostained for SPC and nuclei were counterstained with Hoechst. Scale bar, 30μm **(B)**; 50μm **(D)**.



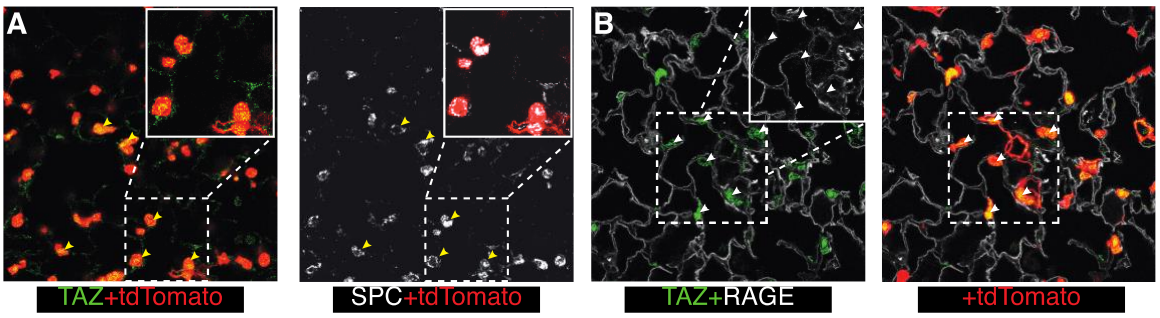
Supplemental Figure 2. A majority of tdTomato^{+} AEC2s is also LysoTracker^{+}. FACS analysis of cells isolated directly from tamoxifen treated SPC-WT-Tm mouse lungs. Epithelial cells were gated based on their surface markers CD45 (-) and EpCAM (+). 95.8% of the total tdTomato^{+}AEC2s are also Lysotracker^{+}(78.4% vs. (78.4+3.41)%).



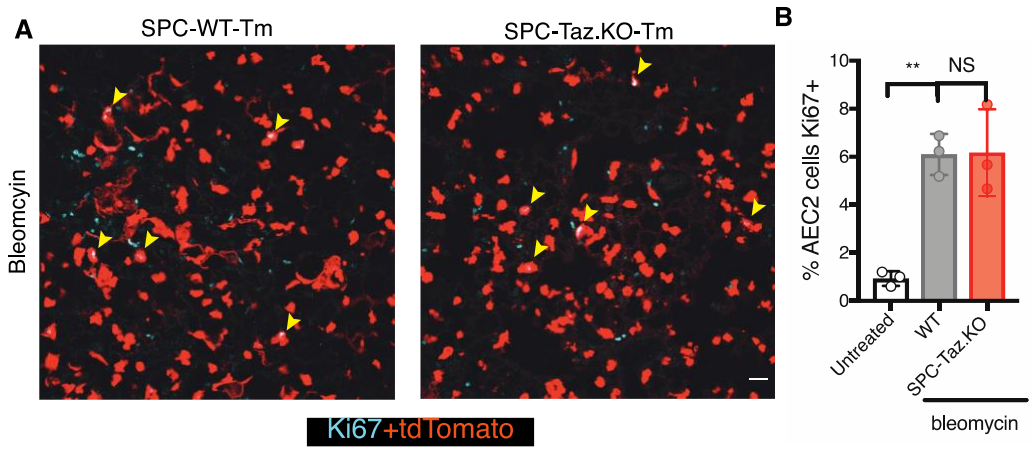
Supplemental Figure 3. Adenovirus-Cre has a high infection efficiency in AEC2s in alveolosphere culture. (A and **B)** To test adenovirus infection efficiency, Rosa26.lsl.tdTomato (SPC-Cre negative) AEC2s were infected with Ad (adenovirus) control or Ad-CRE at the beginning of culture. (**A**) tdTomato^{+}(Red) spheroids in the FL images indicate efficient adenoviral infection and CRE recombinase activity. (**B)** FACS shows that over 90% of the harvested epithelial cells at the end of the Ad-CRE infected culture were tdTomato^{+}. (**C)** Intracellular β-catenin FACS staining shows that 58.7% epithelial cells lost β-catenin expression in the Ad-Cre infected Ctnnb1.fl/fl AEC2 spheroid culture. WT, C57B6. Scale bar, 300μm.



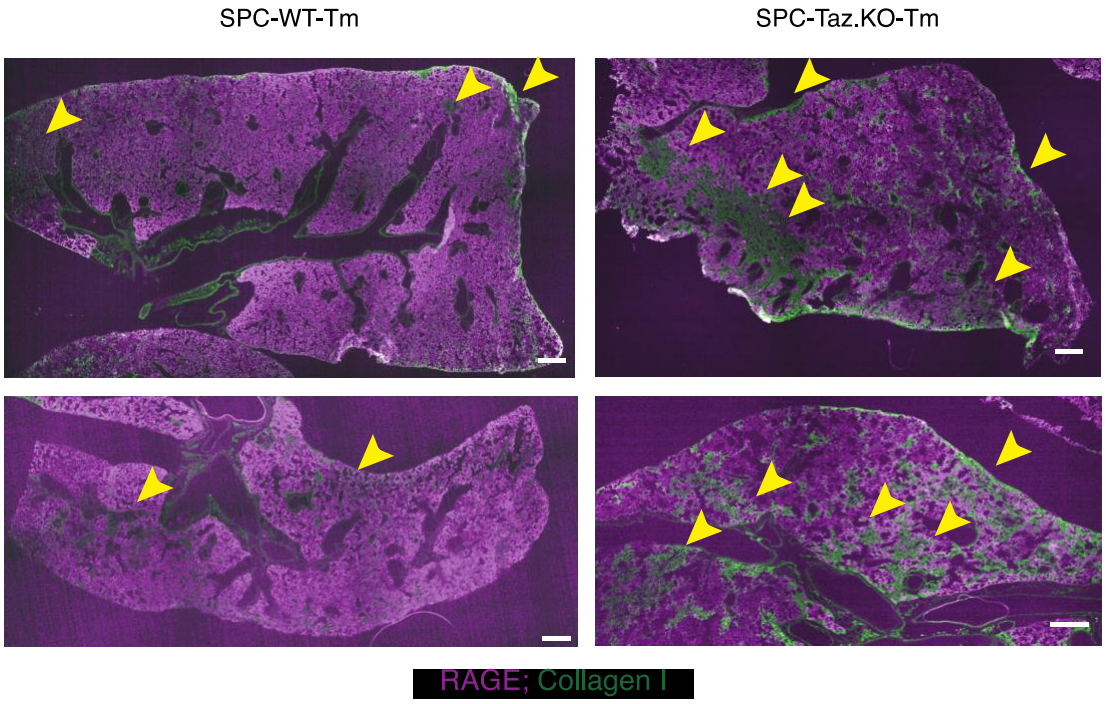
Supplemental Figure 4. **TAZ was efficiently deleted in AEC2s in SPC-Taz.KO-Tm mice. (A)** AEC2s were sorted from tamoxifen treated SPC-WT-Tm and SPC-Taz.KO-Tm mice. *Taz* genomic DNA (gDNA) levels in these sorted cells were evaluated by quantitative PCR (vs. *Tert*); n = 2 WT mice; n = 6 KO mice. (**B)** AEC2s were sorted from these two mouse strains and cultured for spheroid as before. Quantitative RT-PCR on *Taz* expression was performed on cells harvested at the end of the spheroid cultures (relative expression, 6 biological replicates).



Supplemental Figure 5. **TAZ+ tdTomato+ traced AEC cells are SPC^{+}; RAGE^{-} AEC2 cells.** Lungs of SPC-WT-Tm mice were immunostained 10 days after bleomycin instillation as in Figure 5B. Images show co-staining of TAZ with either SPC **(A)** or RAGE **(B).** Insets show high-magnification images**.** TAZ+ tdTomato+ traced AECs cells, indicated by arrowheads, are SPC^{+}and RAGE $^{-}$. Inset in **(B)** shows a closeup image with only the RAGE channel. The locations of those TAZ+ tdTomato+ AEC2s in this image were indicated by white arrowheads.



Supplemental Figure 6. **TAZ deficiency does not affect AEC2 cell proliferation in response to bleomycin injury. (A)** Ki67^{+} tdTomato^{+} cells were indicated by yellow arrowheads to identify actively proliferating AEC2s in representative lung images 10 days after bleomycin administration. (**B)** Quantification shows the percentage (mean ± SD) of Ki67^{+} in total traced AEC2 cells in mouse lungs: untreated SPC-WT-Tm (n = 3 mice with total 2,548 cells counted); bleomycin treated SPC-WT-Tm (n = 3 mice with total 2,175 cells counted); bleomycin treated SPC-Taz.KO-Tm (n = 3 mice with total 2,749 cells counted). $^{**}P < 0.01$; NS: not significant (One-way ANOVA with Tukey's test). Scale bar, 30μm.



Supplemental Figure 7. **TAZ deficiency leads to impaired repair and increased fibrosis in response to bleomycin injury.** FL images show lung lobes of SPC-WT-Tm or SPC-Taz.KO-Tm mice examined 22 days after bleomycin delivery. Lungs were immunostained for RAGE (alveoli/AEC1) and collagen I (fibrotic lesions). Arrows indicate fibrotic lesions with dense collagen deposition. Two lobes with intermediate lesion severities from five individual mouse lungs in each group are shown. Scale bars, 500μm.

Supplemental Table 1: Demographic and baseline clinical characteristics of participants included in plasma sRAGE assessment

	Healthy Control	Non-Progressor	Progressor	P-val.[2]
n	18	63	74	
Age, yr[1]	58 (13)	67.5 (11)	69.2 (10)	0.683
Males, n (%)	10 (56)	52 (83)	56 (76)	0.325
FVC, %predicted		66.3 (21)	66.9 (13)	0.943
DL_{CO}, %predicted		43.9 (15)	37.6 (15)	0.004
GAP Score		4 (2)	5 (1)	0.028
Baseline sRAGE (pg/mL)	2050.1, 1113	1258.9 (535)	970.1 (598)	0.009
sRAGE Change (pg/mL)		+30.2 (260)	–126.7 (246)	0.006

1. Except where specified otherwise, values provided are medians, with interquartile ranges in parentheses
2. All p-values depicted are comparing Progressors Vs. Non-Progressors using Wilcoxon rank

Supplemental Movies: (Movie **S1** and **S2**) "Z stacks" of confocal FL images of whole AEC spheres from SPC-WT-Tm are shown in videos. The spheres, which are tdTomato^{+}(Red), were stained with antibodies for SPC (white) and HOPX (green). Nuclei were counter stained with Hoeschst (blue). The cultures were either treated with control (**S1**) or IWR-1 (**S2**). (Movie **S3** and **S4**) "Z stacks" of confocal FL images of β-catenin+/+ (**S3**) or β-catenin-/- (**S4**) whole AEC spheres are shown in videos. The spheres were stained with antibodies for β-catenin (green), HOPX (Cyan) and SPC (Red). Nuclei were counter stained with Hoeschst (blue).