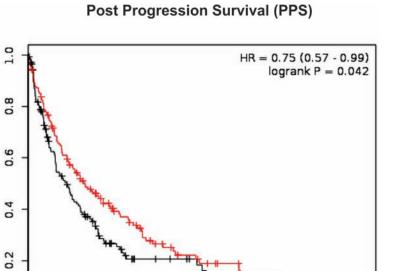
## **SUPPLEMENTARY FIGURES AND TABLES**

Probability

0.0

0



Supplementary Figure S1: Kaplan-Meier curves for comparing post progression survival (PPS) of patients with lung tumors expressing high and low levels of *fibulin-5*.

60

Time (months)

80

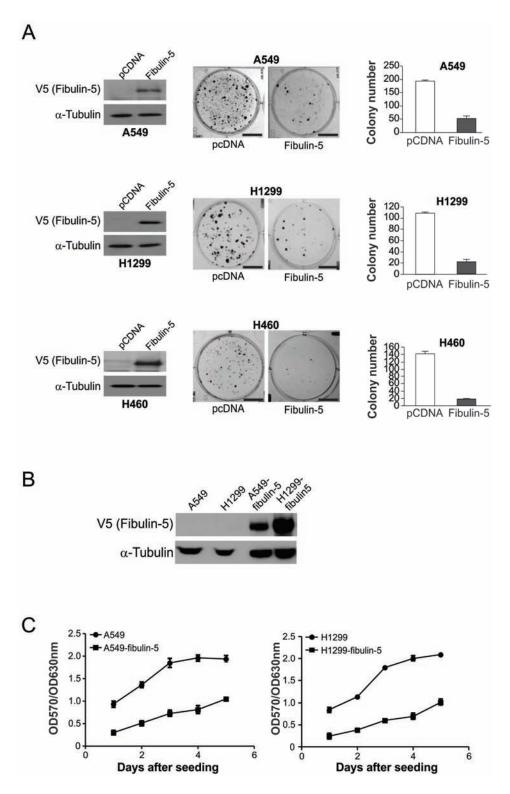
100

120

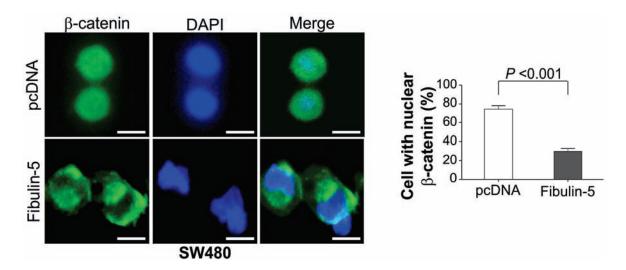
Fibulin-5 high Fibulin-5 low

40

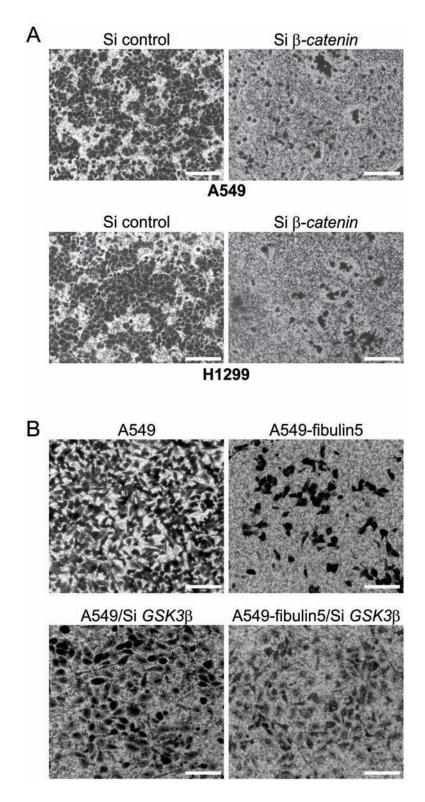
20



**Supplementary Figure S2: Fibulin-5 inhibits lung cancer cell proliferation. A.** H1299, A549, and H460 cells were transfected with V5-tagged fibulin-5 or the control pCDNA vector. *Left*, western blot analysis of fibulin-5 48 hr after transfection; *middle*, cells were plated out 48 hr after fibulin-5 transfection and selected for G418 resistance. Colonies were visualized by crystal violet staining 11 to 14 days later. Scale bars, 1 cm; *right*, quantification of colony numbers. **B.** Western blot analysis of fibulin-5 (V5) in the parental and stable fibulin-5-expressing A549 and H1299 cells. **C.** Equal numbers (3 × 10<sup>3</sup>/well) of parental and stable fibulin-5-expressing A549 and H1299 cells were seeded in 96-well plates. MTT assay was used to analyze cell growth at indicated time after inoculation.



Supplementary Figure S3: Fibulin-5 inhibits β-catenin nuclear translocation. SW480 colorectal cancer cells transfected with *fibulin-5* or the control empty vector were analyzed for β-catenin by immunostaining 48 hr after transfection. *Left*, representative pictures of β-catenin immunostaining with 4' 6-Diamidino-2-phenylindole (DAPI) (blue) for nuclear counterstaining. Scale bar, 5 μm. *Right:* quantification of cells with nuclear β-catenin (P = 0.0008, Student's t test).



Supplementary Figure S4: The effect of fibulin-5 on lung cancer invasion is mediated by  $\beta$ -catenin and GSK-3 $\beta$ . A. Representative pictures of Matrigel invasion analysis of the parental and stable fibulin-5-expressing A549 cells with control or *β*-catenin siRNA transfection. Scale bars, 25 μm. **B.** Representative pictures of Matrigel invasion analysis of invasion of A549 and H1299 cells transfected with the control or  $GSK3\beta$  siRNA. Scale bars, 25 μm.

## Supplementary Table S1. Fibulin-5 and nuclear β-catenin expression in 99 NSCLC

| #  | Sex | Age | Pathology                | Grade | Fibulin-5 expression in tumor | β -catenin expression in tumor |
|----|-----|-----|--------------------------|-------|-------------------------------|--------------------------------|
| 1  | M   | 46  | Squamous cell carcinoma  | I     | _                             | _                              |
| 2  | M   | 46  | Squamous cell carcinoma  | I     | +                             | +                              |
| 3  | M   | 50  | Squamous cell carcinoma  | II    | _                             | +                              |
| 4  | M   | 60  | Squamous cell carcinoma  | II    | _                             | +                              |
| 5  | F   | 64  | Squamous cell carcinoma  | II    | -                             | _                              |
| 6  | M   | 75  | Squamous cell carcinoma  | I     | -                             | _                              |
| 7  | F   | 66  | Squamous cell carcinoma  | I     | -                             | +                              |
| 8  | M   | 62  | Papillary adenocarcinoma | I     | +                             | +                              |
| 9  | M   | 76  | Mucinous adenocarcinoma  | I     | +                             | +                              |
| 10 | F   | 53  | Adenocarcinoma           | I     | +                             | _                              |
| 11 | F   | 55  | Mucinous adenocarcinoma  | I     | +                             | +                              |
| 12 | M   | 42  | Adenocarcinoma           | I     | _                             | _                              |
| 13 | M   | 60  | Papillary adenocarcinoma | I     | _                             | _                              |
| 14 | F   | 43  | Papillary adenocarcinoma | I     | +                             | _                              |
| 15 | M   | 61  | Squamous cell carcinoma  | II    | _                             | +                              |
| 16 | M   | 58  | Squamous cell carcinoma  | II    | _                             | +                              |
| 17 | F   | 52  | Squamous cell carcinoma  | II    | _                             | +                              |
| 18 | F   | 67  | Squamous cell carcinoma  | II    | +                             | +                              |
| 19 | F   | 65  | Squamous cell carcinoma  | II    | +                             | +                              |
| 20 | M   | 78  | Squamous cell carcinoma  | II    | +                             | +                              |
| 21 | M   | 59  | Squamous cell carcinoma  | II    | _                             | _                              |
| 22 | F   | 49  | Squamous cell carcinoma  | II    | _                             | +                              |
| 23 | F   | 72  | Adenocarcinoma           | II    | _                             | _                              |
| 24 | F   | 35  | Adenocarcinoma           | II    | +                             | +                              |
| 25 | M   | 47  | Adenocarcinoma           | II    | _                             | +                              |
| 26 | F   | 54  | Adenocarcinoma           | II    | +                             | +                              |
| 27 | F   | 46  | Adenocarcinoma           | II    | +                             | _                              |
| 28 | F   | 58  | Adenocarcinoma           | II    | +                             | +                              |
| 29 | M   | 47  | Papillary adenocarcinoma | II    | _                             | +                              |
| 30 | F   | 63  | Adenocarcinoma           | II    | _                             | +                              |
| 31 | F   | 37  | Adenocarcinoma           | II    | _                             | +                              |
| 32 | M   | 47  | Mucinous adenocarcinoma  | II    | _                             | _                              |
| 33 | M   | 53  | Mucinous adenocarcinoma  | II    | _                             | -                              |
| 34 | F   | 55  | Mucinous adenocarcinoma  | II    | _                             | -                              |
| 35 | M   | 48  | Adenocarcinoma           | II    | _                             | _                              |
| 36 | M   | 56  | Squamous cell carcinoma  | II    | +                             | +                              |

(Continued)

| #  | Sex | Age | Pathology                | Grade | Fibulin-5 expression in tumor | β -catenin expression<br>in tumor |
|----|-----|-----|--------------------------|-------|-------------------------------|-----------------------------------|
| 37 | F   | 63  | Adenocarcinoma           | II    | -                             | +                                 |
| 38 | F   | 58  | Adenocarcinoma           | II    | -                             | _                                 |
| 39 | F   | 67  | Adenocarcinoma           | II    | +                             | +                                 |
| 40 | M   | 46  | Adenocarcinoma           | II    | -                             | _                                 |
| 41 | M   | 59  | Papillary adenocarcinoma | II    | -                             | -                                 |
| 42 | M   | 40  | Squamous cell carcinoma  | II    | _                             | _                                 |
| 43 | F   | 32  | Adenocarcinoma           | II    | -                             | +                                 |
| 44 | M   | 40  | Squamous cell carcinoma  | II    | _                             | _                                 |
| 45 | M   | 57  | Squamous cell carcinoma  | II    | +                             | +                                 |
| 46 | M   | 72  | Squamous cell carcinoma  | III   | +                             | +                                 |
| 47 | M   | 58  | Squamous cell carcinoma  | III   | _                             | -                                 |
| 48 | M   | 53  | Squamous cell carcinoma  | III   | +                             | +                                 |
| 49 | M   | 41  | Squamous cell carcinoma  | III   | -                             | -                                 |
| 50 | M   | 54  | Squamous cell carcinoma  | III   | _                             | +                                 |
| 51 | M   | 50  | Squamous cell carcinoma  | III   | +                             | +                                 |
| 52 | F   | 49  | Squamous cell carcinoma  | III   | _                             | +                                 |
| 53 | M   | 51  | Squamous cell carcinoma  | III   | +                             | -                                 |
| 54 | M   | 65  | Squamous cell carcinoma  | III   | -                             | +                                 |
| 55 | M   | 72  | Squamous cell carcinoma  | III   | _                             | +                                 |
| 56 | M   | 51  | Squamous cell carcinoma  | III   | _                             | _                                 |
| 57 | M   | 53  | Squamous cell carcinoma  | III   | +                             | -                                 |
| 58 | M   | 56  | Squamous cell carcinoma  | III   | -                             | +                                 |
| 59 | M   | 56  | Squamous cell carcinoma  | III   | +                             | -                                 |
| 60 | M   | 61  | Squamous cell carcinoma  | III   | -                             | +                                 |
| 61 | M   | 66  | Squamous cell carcinoma  | III   | -                             | +                                 |
| 62 | F   | 45  | Adenocarcinoma           | I     | +                             | +                                 |
| 63 | M   | 47  | Squamous cell carcinoma  | III   | -                             | _                                 |
| 64 | M   | 57  | Squamous cell carcinoma  | III   | _                             | +                                 |
| 65 | M   | 57  | Squamous cell carcinoma  | III   | +                             | _                                 |
| 66 | M   | 67  | Squamous cell carcinoma  | II    | _                             | +                                 |
| 67 | F   | 41  | Squamous cell carcinoma  | III   | _                             | +                                 |
| 68 | F   | 51  | Squamous cell carcinoma  | II    | +                             | +                                 |
| 69 | F   | 60  | Squamous cell carcinoma  | III   | _                             | _                                 |
| 70 | M   | 61  | Squamous cell carcinoma  | III   | _                             |                                   |
| 71 | M   | 61  | Squamous cell carcinoma  | III   | +                             |                                   |
| 72 | M   | 61  | Squamous cell carcinoma  | III   | +                             | _                                 |

(Continued)

| #  | Sex | Age | Pathology               | Grade | Fibulin-5 expression in tumor | β -catenin expression in tumor |
|----|-----|-----|-------------------------|-------|-------------------------------|--------------------------------|
| 73 | F   | 71  | Squamous cell carcinoma | II    | +                             | +                              |
| 74 | F   | 71  | Squamous cell carcinoma | III   | _                             | _                              |
| 75 | F   | 71  | Squamous cell carcinoma | III   | +                             | _                              |
| 76 | F   | 49  | Adenocarcinoma          | III   | _                             | +                              |
| 77 | M   | 59  | Adenocarcinoma          | III   | _                             | +                              |
| 78 | F   | 50  | Adenocarcinoma          | III   | +                             | -                              |
| 79 | M   | 35  | Adenocarcinoma          | III   | _                             | -                              |
| 80 | M   | 62  | Adenocarcinoma          | III   | _                             | +                              |
| 81 | F   | 50  | Squamous cell carcinoma | III   | _                             | +                              |
| 82 | F   | 54  | Squamous cell carcinoma | III   | +                             | +                              |
| 83 | M   | 64  | Squamous cell carcinoma | III   | _                             | -                              |
| 84 | M   | 65  | Squamous cell carcinoma | III   | +                             | +                              |
| 85 | F   | 53  | Squamous cell carcinoma | III   | +                             | -                              |
| 86 | M   | 63  | Squamous cell carcinoma | III   | _                             | +                              |
| 87 | M   | 62  | Squamous cell carcinoma | III   | _                             | +                              |
| 88 | M   | 62  | Squamous cell carcinoma | III   | _                             | _                              |
| 89 | M   | 59  | Adenocarcinoma          | III   | +                             | _                              |
| 90 | M   | 46  | Squamous cell carcinoma | III   | +                             | +                              |
| 91 | M   | 74  | Squamous cell carcinoma | IV    | _                             | +                              |
| 92 | M   | 60  | Squamous cell carcinoma | II    | _                             | +                              |
| 93 | M   | 54  | Adenocarcinoma          | III   | _                             | -                              |
| 94 | M   | 67  | Adenocarcinoma          | III   | _                             | +                              |
| 95 | F   | 56  | Adenocarcinoma          | III   | _                             | _                              |
| 96 | F   | 65  | Adenocarcinoma          | III   | +                             | _                              |
| 97 | M   | 51  | Squamous cell carcinoma | III   | _                             | +                              |
| 98 | M   | 45  | Squamous cell carcinoma | III   | _                             | +                              |
| 99 | F   | 39  | Adenocarcinoma          | III   | _                             | +                              |