

TLR7 and 8 are expressed in human bronchial epithelial cells but not in alveolar epithelial cells in lung emphysema. TLR7 (A, C) and TLR8 (B, D) protein expression was analyzed in alveolar epithelium (A, B) and in bronchial epithelium (C, D) of lung tissues from emphysema patients, by immunohistochemical labeling of paraffin-embedded tissues, as described in the materials and methods (Original magnification, x40).

## Supplemental Figure 2



TLR3 and 4 stimulation lead to  $I\kappa B\alpha$  phosphorylation, but not degradation. A549 cells were untreated or treated with Poly I:C (10 µg/ml) (A, B), or LPS (10 µg/ml) C, D) for the indicated periods of time. The cell lysates were analyzed by immunoblot with anti-phospho-specific  $I\kappa B\alpha$ , anti-  $I\kappa B\alpha$ , and anti-Actin antibodies (A, C). Quantification of the bands was realized using Image J software. Histograms represent the intensity of each band observed in the blots at the indicated time point and are expressed in arbitrary units normalized to Actin intensity (relative intensity) both for phospho-I $\kappa$ B $\alpha$  and I $\kappa$ B $\alpha$  (B, D). Results are representative of three independent experiments.





LD50 was determined as the drug concentration giving 50% reduced Alamar blue. Data represent mean values +/- SD from 3 independent experiments.



TLR4 but not TLR3 stimulation induces chemoresistance of lung tumor cells depending of the cell type. A549 (A, B) or SK-MES cells (C, D) were cultured in 6 well plates with or without Poly I:C or LPS (added at days 0, 3, 6 and 9), and were treated or not with cycloheximide, cisplatine, carboplatine, doxorubicine, or Navelbine at day 12. The colony number was determined after Crystal Violet coloration at the day 15 and cell viability was analyzed by the surviving fraction. The surviving fraction (C, D) was calculated as follow: SF (Surviving Fraction)= Number of colonies after chemotherapy treatment/(number of cells seeded at day 0 x PE) x 100. PE (Plating Efficiency) = number of colonies/number of cells seeded at day 0. Data represent

mean values +/- SD from 3 independent experiments (\*p < 0.05; \*\*p < 0.01, \*\*\*p < 0.001, Student test).



**TaqMan LDA analysis is reproducible.** A549 or SK-MES cells were cultured for 6h in the presence of Loxoribine. Total RNA was extracted and analyzed for the expression of 182 genes using Taq Man Low Density Array technology. All genes with a value greater than 35 (CT) were excluded from the analysis. The ΔCT values for the expression of all genes were calculated by subtraction of the CT value of the 18S housekeeping gene. The ΔΔCT values were obtained by subtraction of the ΔCT values for unstimulated cells from those obtained for TLR7- or 8-stimulated cells. The fold increase (arbitrary unit) was obtained by  $2^{-\Delta \Delta CT}$ . Values were then clustered using the Genesis Software. Data represents gene modulation of three independent experiments.

Supplemental Figure 6



High correlation between human primary lung cancer cells from the same histological type. Total RNA was extracted from primary tumor cells of 3 ADC patients (P33, P84, P97, (A)) and of 3 SCC patients (P23, P25, P31, (B)) and the expression of 182 genes was assayed using Taq Man Low Density Array technology after retrotranscription. All genes with a CT value greater than 35CT were excluded from the analysis. The  $\Delta$ CT values for gene expression were calculated by substraction of the CT value of the 18S housekeeping gene. The  $\Delta$ CT value for each gene was compared between each patient of the same histological type, to determine the correlation index.

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