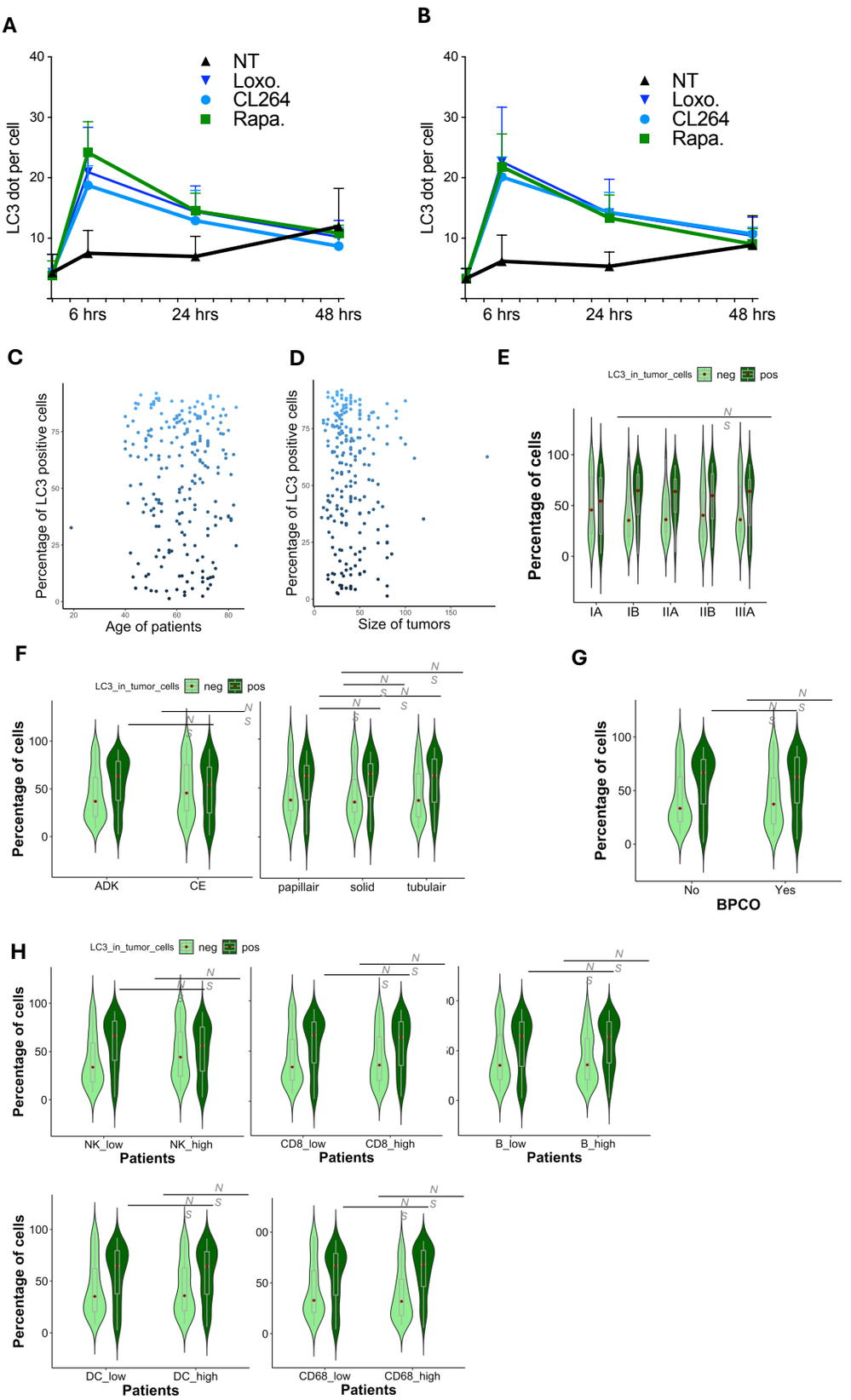

Figure S1 Analysis of autophagy induction upon TLR7 stimulation in SK-MES and LLC lung tumor cell lines and Correlation analysis of autophagy levels in NSCLC tumor cells with clinical data and immune infiltration. (A) SK-MES were treated or not (NT) with an autophagy inducer, the rapamycin (rapa, 1µM) or by synthetic TLR7 agonists, CL264 (1mM) and loxoribin (Loxo, 1mM). The graph represents the quantification of the LC3 dots per cell (analyzed using anti-LC3 antibody) in each condition with different kinetics: 6, 24, and 48h post-treatments. The experiment was performed three times. (B) LLC were treated or not (NT) with an autophagy inducer, the rapamycin (rapa, 1µM) or by synthetic TLR7 agonists, CL264 (1mM) and loxoribin (Loxo, 1mM). The graph represents the quantification of the LC3 dots per cell (analyzed using anti-LC3 antibody) in each condition with different kinetics: 6, 24, and 48h post-treatments. The experiment was performed three times. (C and D). Evaluation of the percentage of lung tumor cells positive for LC3 in function of the age of the NSCLC patient (C) and the size of the tumor (D). (E-H). Evaluation of the proportion of lung tumor cells positive or negative for LC3 in function of the stage of cancer (E), the phenotype of the tumor (F), the BPCO status (G) or, the immune cells infiltration (H). For immune infiltration, high versus low patients was defined as the median of density for each immune cell.

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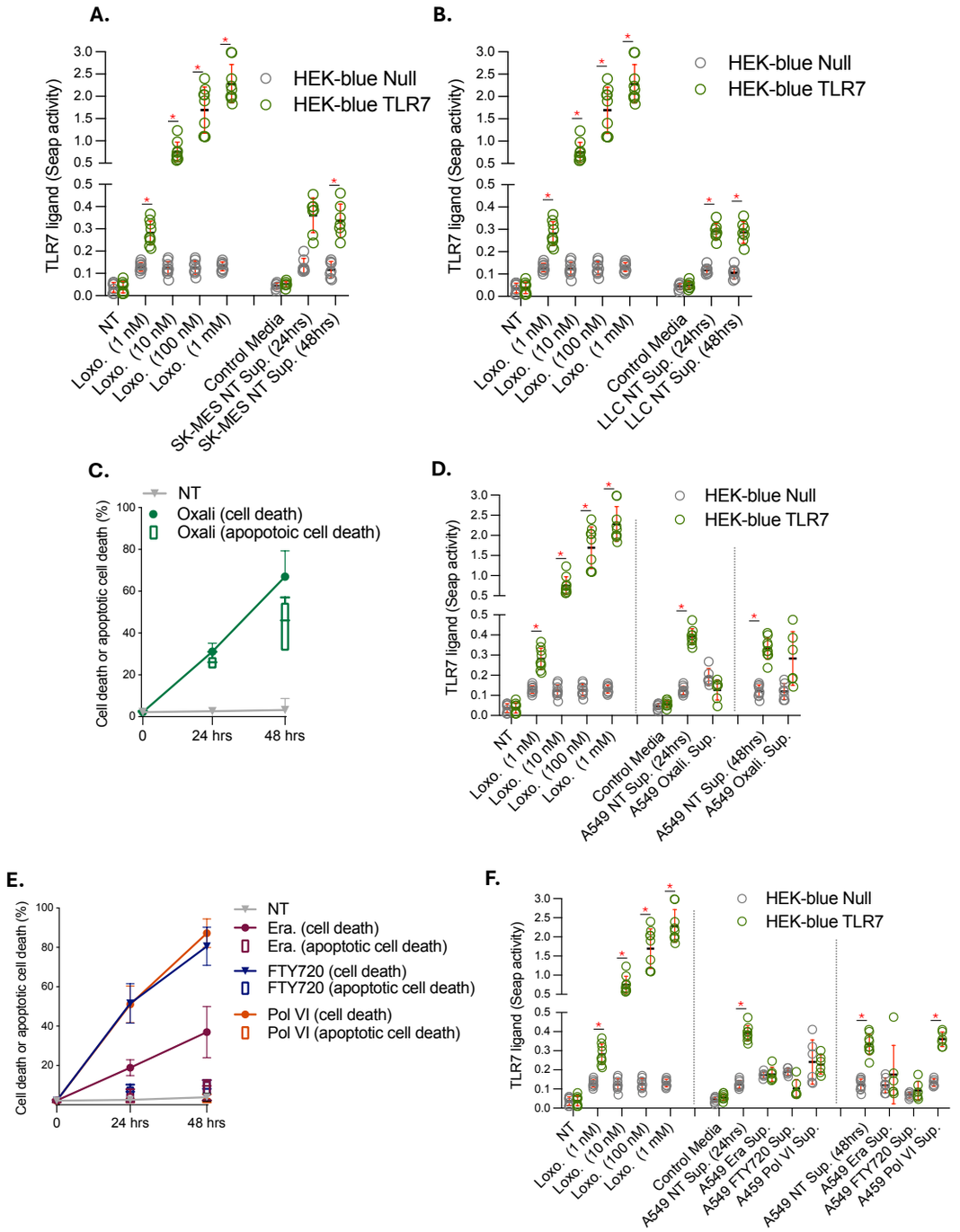

Figure S2 Investigation into the presence of TLR7 ligands in various lung tumor cell lines and determine the impact of cell death on its released. (A) Measurement of the TLR7 ligands abundance following the treatment or not (NT) with different doses of TLR7 agonists (Loxo., 1nM, 10nM, 100nM, and 1mM), or after the incubation with a culture medium alone (Control media), or with supernatant from SK-MES in culture for 24h (A549 NT Sup. (24hrs)) or 48h (A549 NT Sup. (48hrs)). The experiment was performed three times in triplicates. (B) Similar experiment as in A, but the supernatant from LLC cells lines. (C) Graph representing the mortality rate (Live dead +) as well as the apoptotic rate (Annexin V+/ PI-) of A549 treated cells or not (NT) with oxaliplatin (Oxali., 200µM) for 24h and 48h. The experiment was performed three times in triplicates. (D) Measurement of the abundance of TLR7 ligands through the measurement of the Sepa activity of HEK-blue TLR7 cells versus HEK-blue Null cells following the treatment or not (NT) with different doses of TLR7 agonists (Loxo. , 1nM, 10nM , 100nM, and 1mM), or after the incubation with a culture medium alone (Control media), or with supernatant from A549 in culture for 24h (A549 NT Sup. (24hrs)) or 48h (A549 NT Sup. (48hrs)) or with supernatant from A549 treated with oxaliplatin (Oxali., 200µM) (A549 Oxali Sup.) for 24h and 48h. The experiment was performed three times in triplicates. (E) Graph representing the percentage of dead (Live dead +) and apoptotic (Annexin V+/ PI-) lung tumor cells following treatment with the different cell death inducers: ferroptosis (erastin, era.,15µM), necroptosis (FTY720, 10µM) and pyroptosis (polyphillin VI, pol VI., 8µM) at 24 and 48h post-treatment. The experiment was performed three times in triplicates. (F) Measurement of the abundance of TLR7 ligands through the measurement of the Sepa activity of HEK-TLR7 cells versus HEK-Null cells following the treatment or not (NT) with different doses of TLR7 agonists (Loxo. , 1nM, 10nM, 100nM, and 1mM), or after the incubation with a culture medium alone (Control media), or with supernatant from A549 in culture for 24h (A549 NT Sup. (24hrs)) or 48h (A549 NT Sup. (48hrs)) or with supernatant from A549 treated with the different cell death inducers for 24h and 48h. The experiment was performed three times in triplicates. Student's *t*-test, * : p<0.05.

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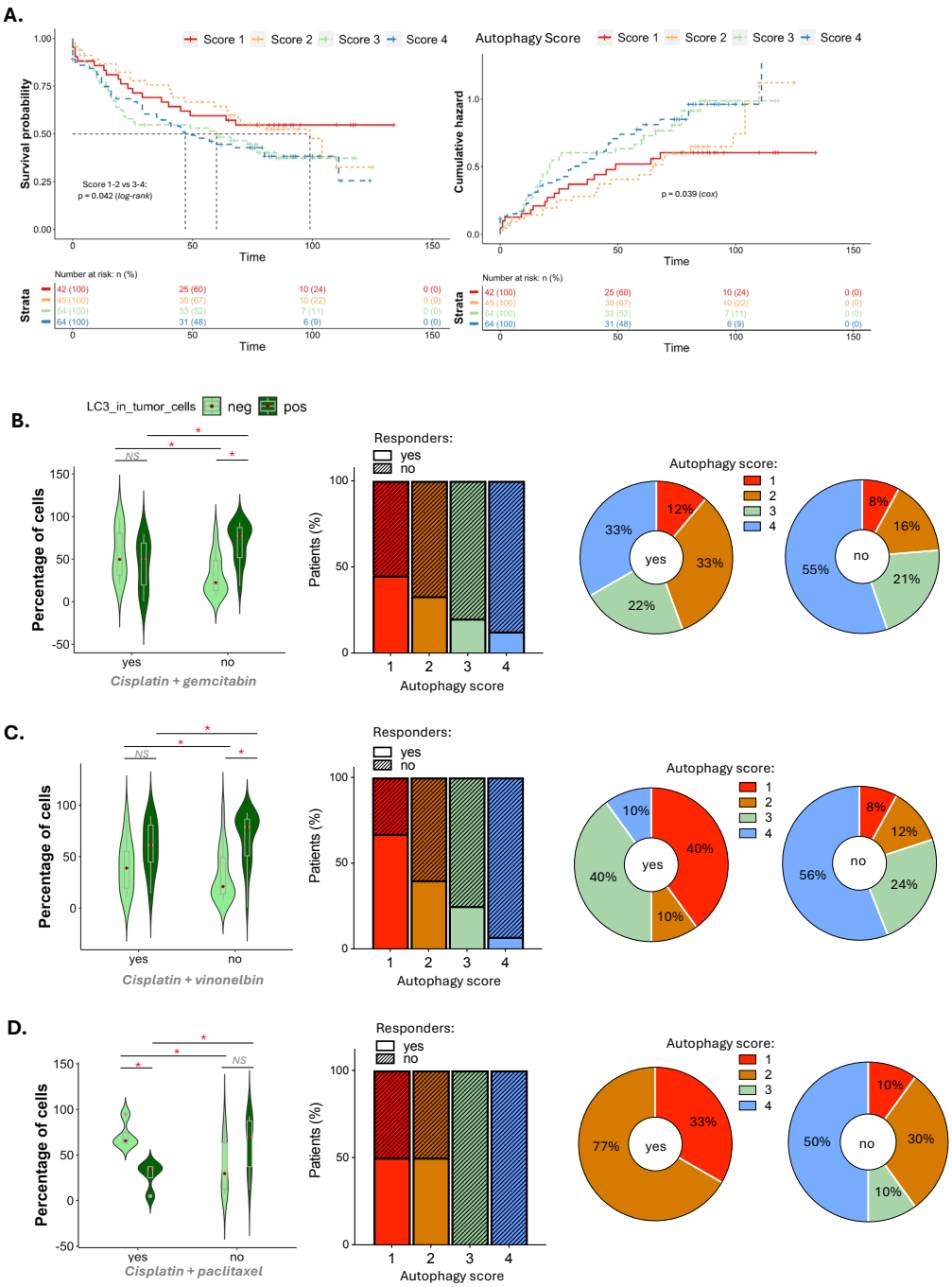

Figure S3 Impact of LC3 level in lung tumor cells on the overall survival and the resistance to neoadjuvant chemotherapies combinations in NSCLC patients. (A) Left: Kaplan Meier representing the survival probability of patients over time based on their autophagy score (from 1, the less autophagic, to 4, the more autophagic NSCLC patients). Right: Kaplan Meier describing the cumulative hazard over time based on the autophagy score of NSCLC patients. (B) Left: Graph representing the percentage of LC3 positive versus LC3 negative lung tumor cells in NSCLC patients treated with neoadjuvant chemotherapies in function of their responsiveness to the combination of cisplatin plus gemcitabine. Middle: Distribution of the proportion of NSCLC patients (n=43) who respond (yes) versus do not respond to the chemotherapies combination (no) in function of their autophagy score. Right: Proportion of the different autophagy scores of NSCLC patients between responders (yes) versus non-responders to the chemotherapies combination (no). (C) Similar experiment as in A but patients has been treated by the combination of cisplatin plus vinorelbine (n=35). (D) Similar experiment as in A but patients has been treated by the combination of cisplatin plus paclitaxel (n=14).

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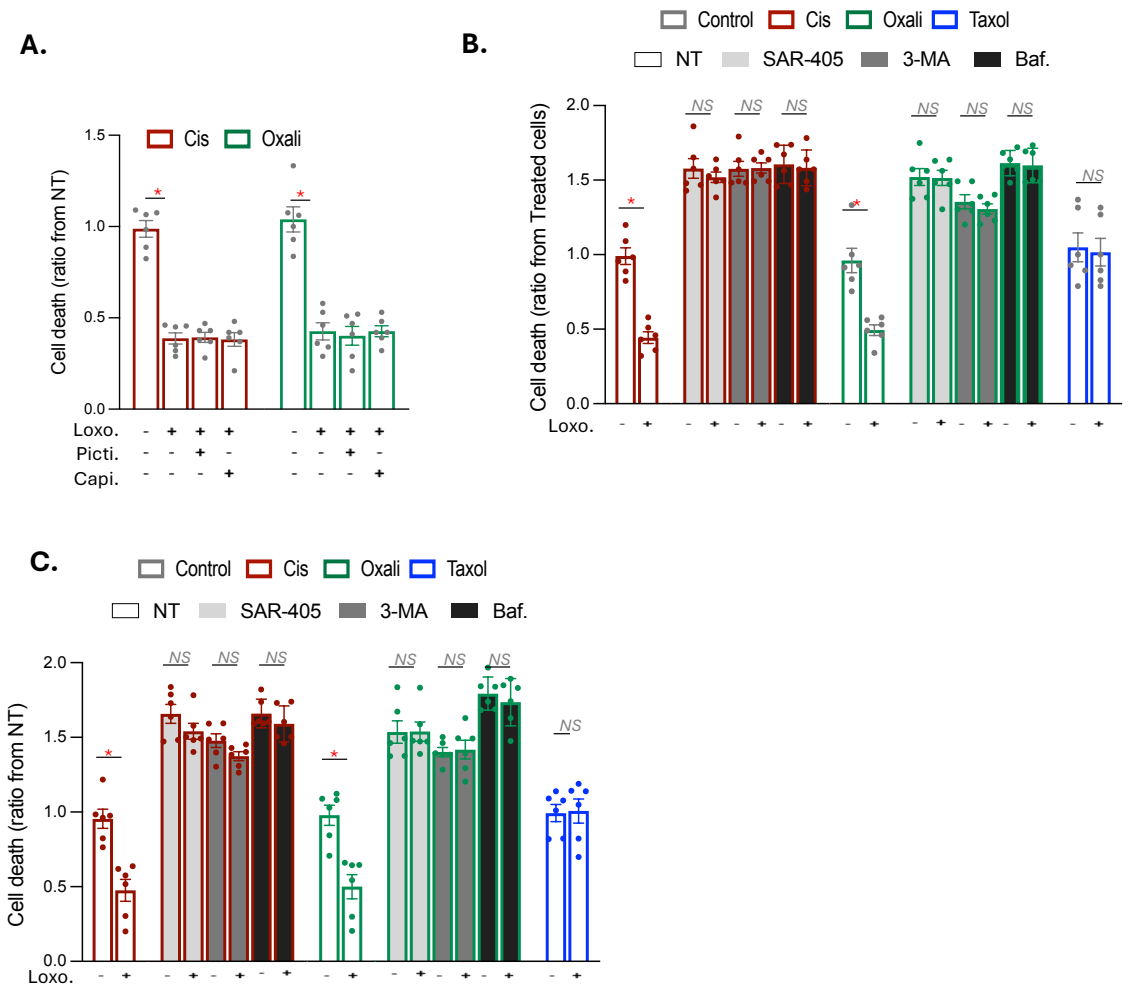

Figure S4 Impact of autophagy on the resistance to chemotherapies in various lung tumor cell lines. (A) Graph showing the mortality ratio of the different combination treatments from that of chemotherapy alone for each chemotherapy at 48 hours post-treatment. In addition to treatment with cisplatin (Cis, 50µM), or oxaliplatin (200µM), A459 cells were treated or not with a synthetic TLR7 agonist (Loxo., 1mM) alone or with either an inhibitor of a pan AKT inhibitors (Capivasertib, Capi., 8nM), or a pan type 1 PI3K inhibitor (Pictilisib, picti., 3nM), both targeting the type 1 PI3K. The experiment was performed at least three times in triplicates. (B) Graph showing the mortality ratio (ration from NT) of the different combination of treatments at 48 hours. In addition to treatment with chemotherapies, SK-MES were treated or not with a synthetic TLR7 agonist (Loxo., 1mM) alone or with different autophagy inhibitors, 3-methyladenine (3-MA., 10mM), wortmannin (wort., 100nM), SAR405 (10µM) or bafilomycin (baf., 100nM). The experiment was performed at least three times in triplicates. (C) Similar experiment as in (D), using LLC lung tumor cell lines. The experiment was performed at least three times in triplicates. Student's *t*-test, * : p<0.05.

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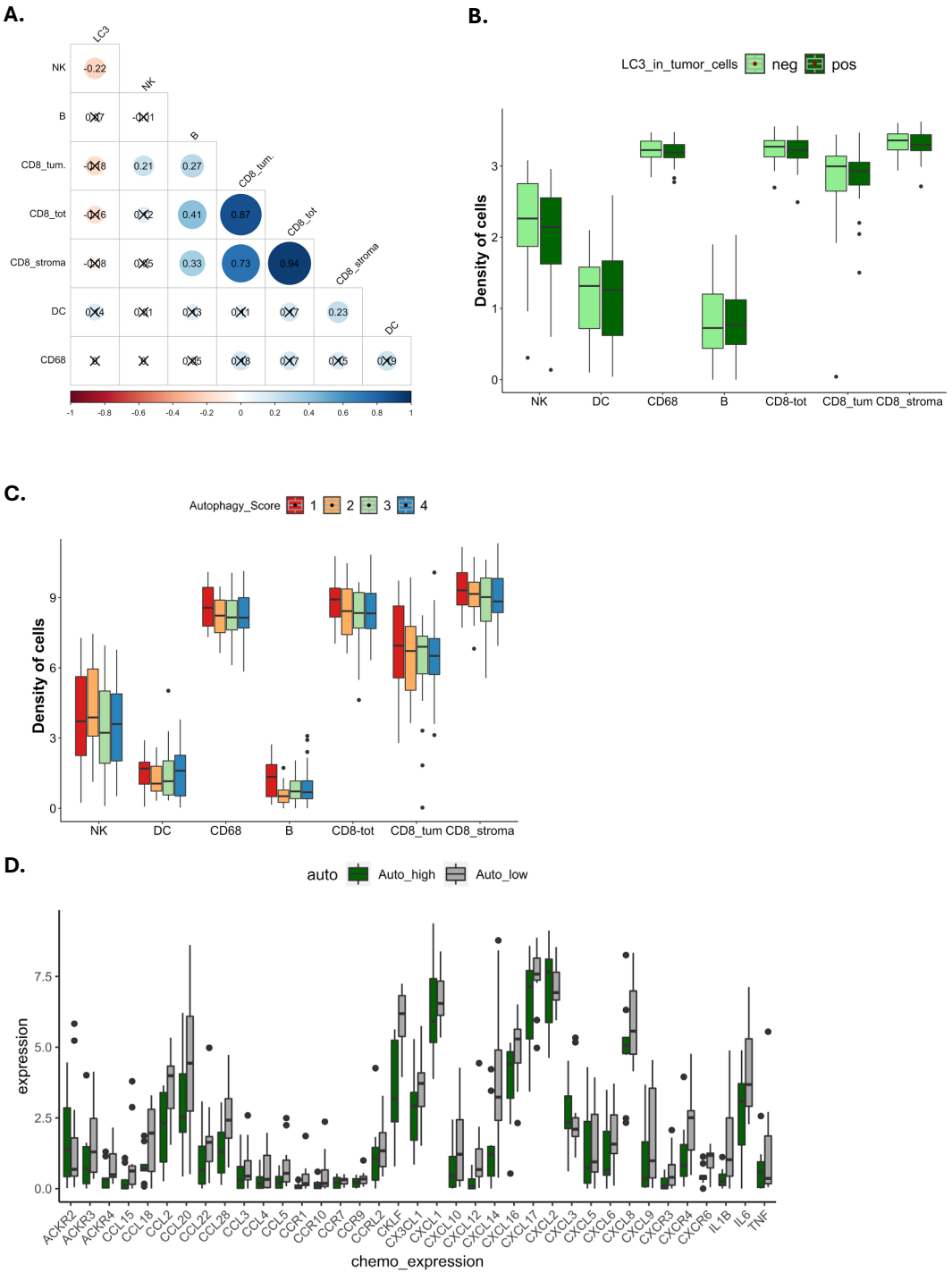

Figure S5 Effect of autophagy level in lung tumor cells on the infiltration of immune cells in NSCLC patients. (A) Study of correlation between the LC3 expression in NSCLC tumor cells and the infiltration within the tumor of the main immune cell types (NK, CD8 T cells, B cells, DC, and macrophages (CD68)) using our cohort 1. From the more correlated in blue to the more inversely correlated in red. Cross means no significant correlation. (B) Measurement of the density of each immune cell type infiltrated in function of whether the NSCLC patients are LC3 low versus LC3 high. (C) Measurement of the density of each immune cell type infiltrated in function of the autophagy score of the NSCLC patients. (D) Study the correlation between genes expression encoding cytokines, chemokines, and their receptors with autophagy genes in malignant cells using GSE111907 datasets.

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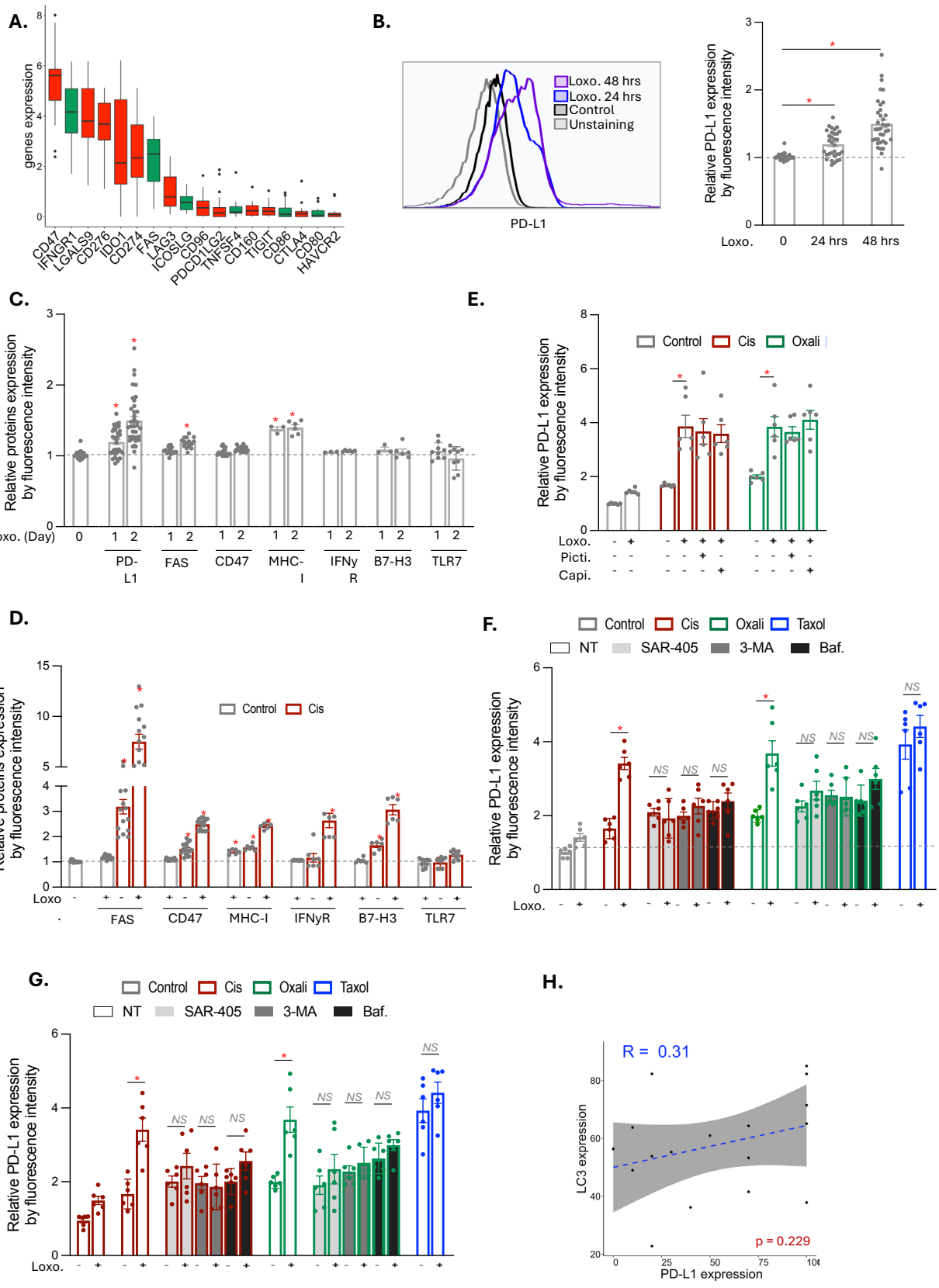

Figure S6 Effect of TLR7 synthetic agonist alone or in combination with cisplatin on the expression of molecules impacting the immune phenotype of the lung tumor cells. (A) Graph representing the gene expression of immune checkpoints in NSCLC tumor. The gene expression was evaluated using sorted NSCLC malignant cells (GSE-111907 dataset). In red, genes coding for proteins which exert pro-tumoral effects, and in green, genes coding for proteins which exert pro-tumoral effects. (B) Left: histogram of PD-L1 fluorescence intensity by flow cytometry in TLR7 synthetic agonist-treated A549 cells (Loxo., 1mM) for 24h and 48h. Right: Graph describing the ratio of the relative PD-L1 expression by fluorescence intensity measured by flow cytometry in TLR7 synthetic agonist-treated A549 cells (Loxo., 1mM) from the value observed in non-treated cells, for 24h and 48h. The experiment was performed three times in triplicates. (C) Graph describing the ratio of relative proteins expression by fluorescence intensity measured by flow cytometry of various molecules in TLR7 synthetic agonist-treated A549 cells (Loxo., 1mM) from the value observed in non-treated cells, for 24h and 48h. The experiment was performed at least three times in triplicates. (D) Graph representing the ratio of relative proteins expression by fluorescence intensity measured by flow cytometry of various molecules in TLR7 synthetic agonist-treated A549 cells alone (Loxo. 1mM), or in combination with cisplatin (Cis., 50µM) (Loxo + cis), from the value observed in non-treated cells, at 48h post-treatment. The experiment was performed at least three times in triplicates. (E) Graph representing the ratio of the relative PD-L1 expression by fluorescence intensity measured by flow cytometry of the different combination treatments from that of cisplatin alone at 48 hours post-treatment. In addition to treatment with cisplatin (Cis, 50µM), cells were treated or not with a synthetic TLR7 agonist (Loxo., 1mM) alone or in combination with either an inhibitor of a pan AKT inhibitors (Capivasertib, Capi., 8nM), or a pan type 1 PI3K inhibitor (Pictilisib, picti., 3nM), both targeting the type 1 PI3K. The experiment was performed at least three times in triplicates. (F) Graph representing the ratio of the relative PD-L1 expression (from NT condition) measured by flow cytometry in TLR7 synthetic agonist-treated SK-MES cells alone (Loxo. 1mM) or in combination with different chemotherapies at 48h post-treatment. The chemotherapies used are cisplatin (Cis., 50µM), oxaliplatin (Oxali., 200µM), and taxol (10µM). In addition, different autophagy inhibitors (3-MA., 10mM, and SAR405, 10µM, Baf., 100nM) or type 1 PI3K inhibitor (Pictilisib, picti., 3nM) were added to these combinatory treatments. The experiment was performed at least three times in triplicates. (G) Similar experiment as in E, using LLC lung tumor cell lines. The experiment was performed at least three times in triplicates. (H) Correlation of LC3 expression in lung tumor cells with PD-L1 expression on these same cells, using a retrospective cohort of NSCLC patients (cohort 3). Student's *t*-test, * : p<0.05.

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