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Title	4-Nonylphenol effects on rat testis and sertoli cells determined by spectrochemical techniques coupled with chemometric analysis
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Creators	Duan, Peng, Liu, Bisen, Medeiros-De-morais, Camilo De Ielis, Zhao, Jing, Li, Xiandong, Tu, Jian, Yang, Weiyinxue, Chen, Chunling, Long, Manman, Feng, Xiaobing, Martin, Francis L and Xiong, Chengliang

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Figure legends

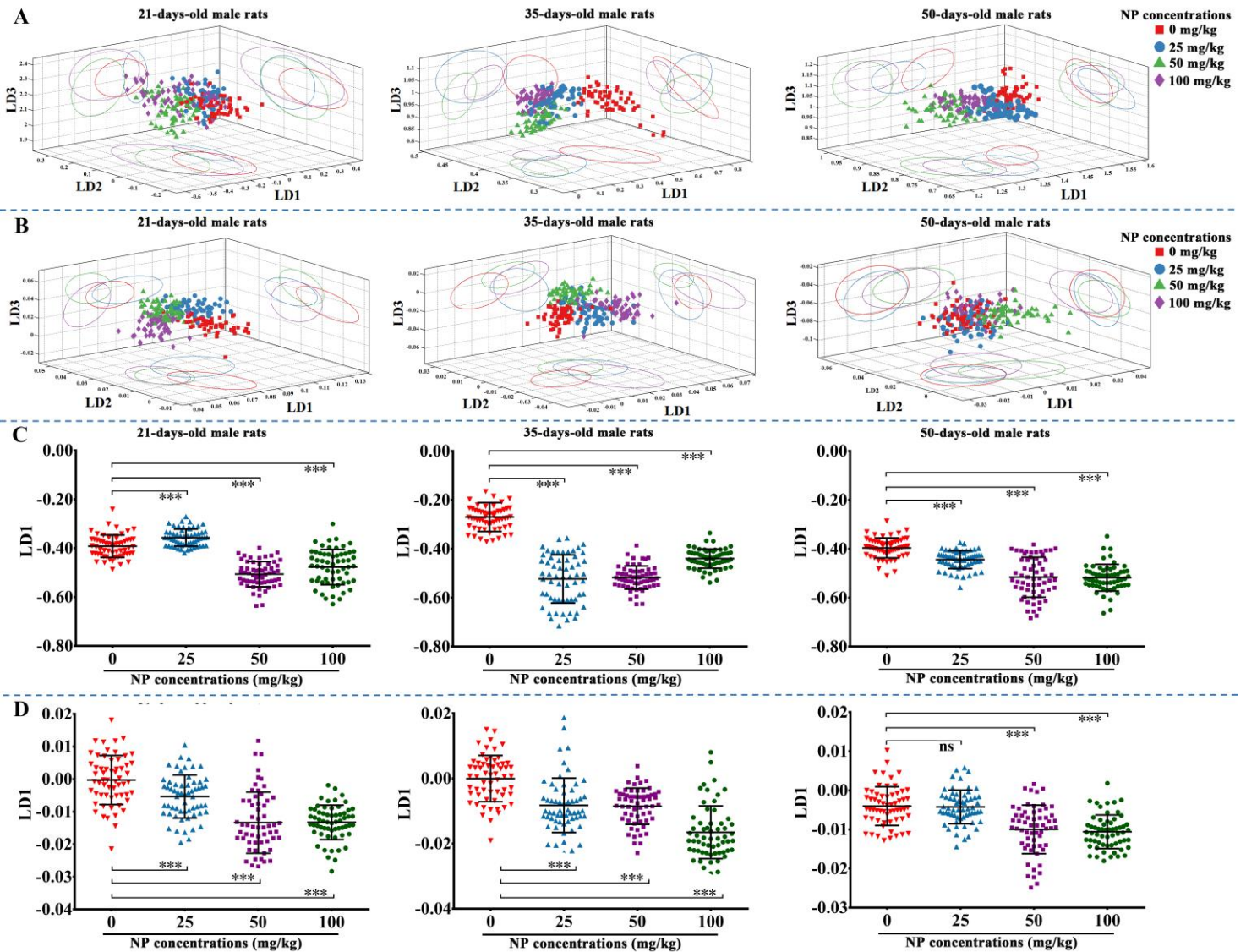
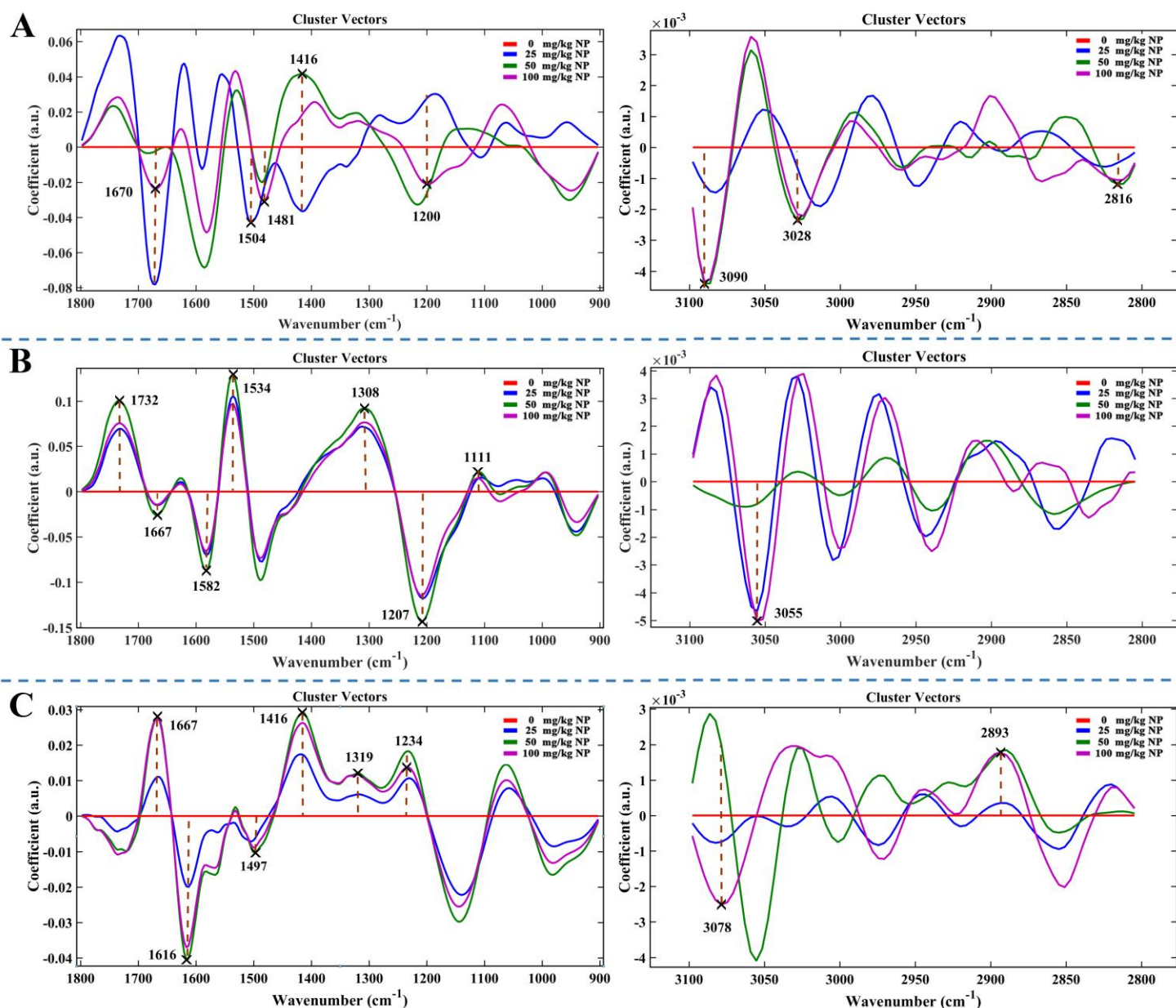
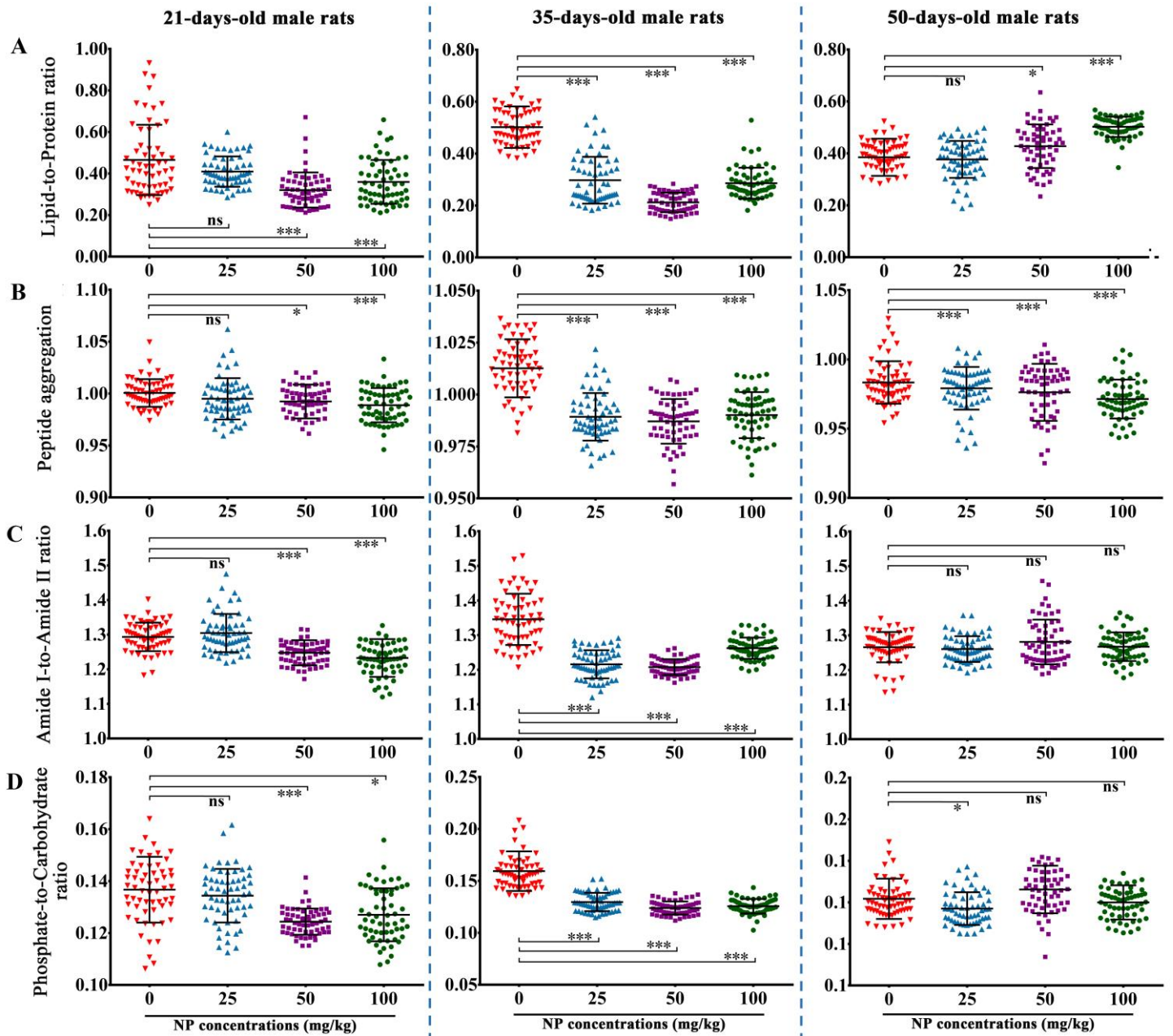


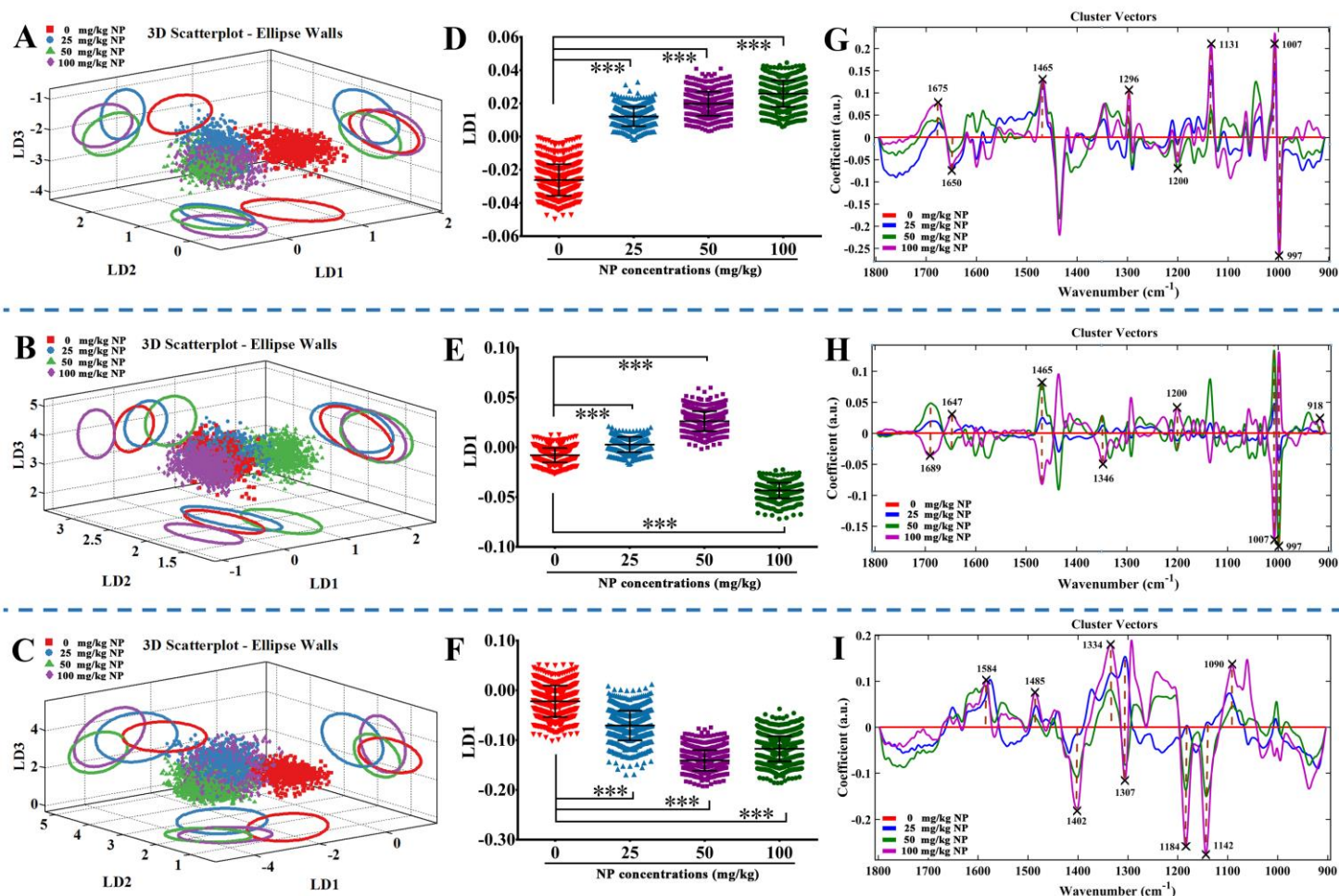
Figure 1. PCA-LDA of ATR-FTIR spectral data extracted from the testicular cells of rats exposed to 4-Nonylphenol (NP) at each concentration vs. control. Three-dimensional (3-D) PCA-LDA scores plots for ATR-FTIR spectra regions of 1800-900 cm^{-1} (A) and of 3200-2800 cm^{-1} (B). Linear discriminant 1 (LD1) scatter plots from PCA-LDA for ATR-FTIR spectra regions of 1800-900 cm^{-1} (C) and of 3200-2800 cm^{-1} (D). Confidence ellipsoids (90%) were drawn in each 3D scores plot. The data of each LD1 scatter plot is represented as mean \pm standard deviations. $n=6$ for each group. Significance of category segregation was determined using one-way ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test, *** $P < 0.001$ versus the control group (0 mg/kg NP).



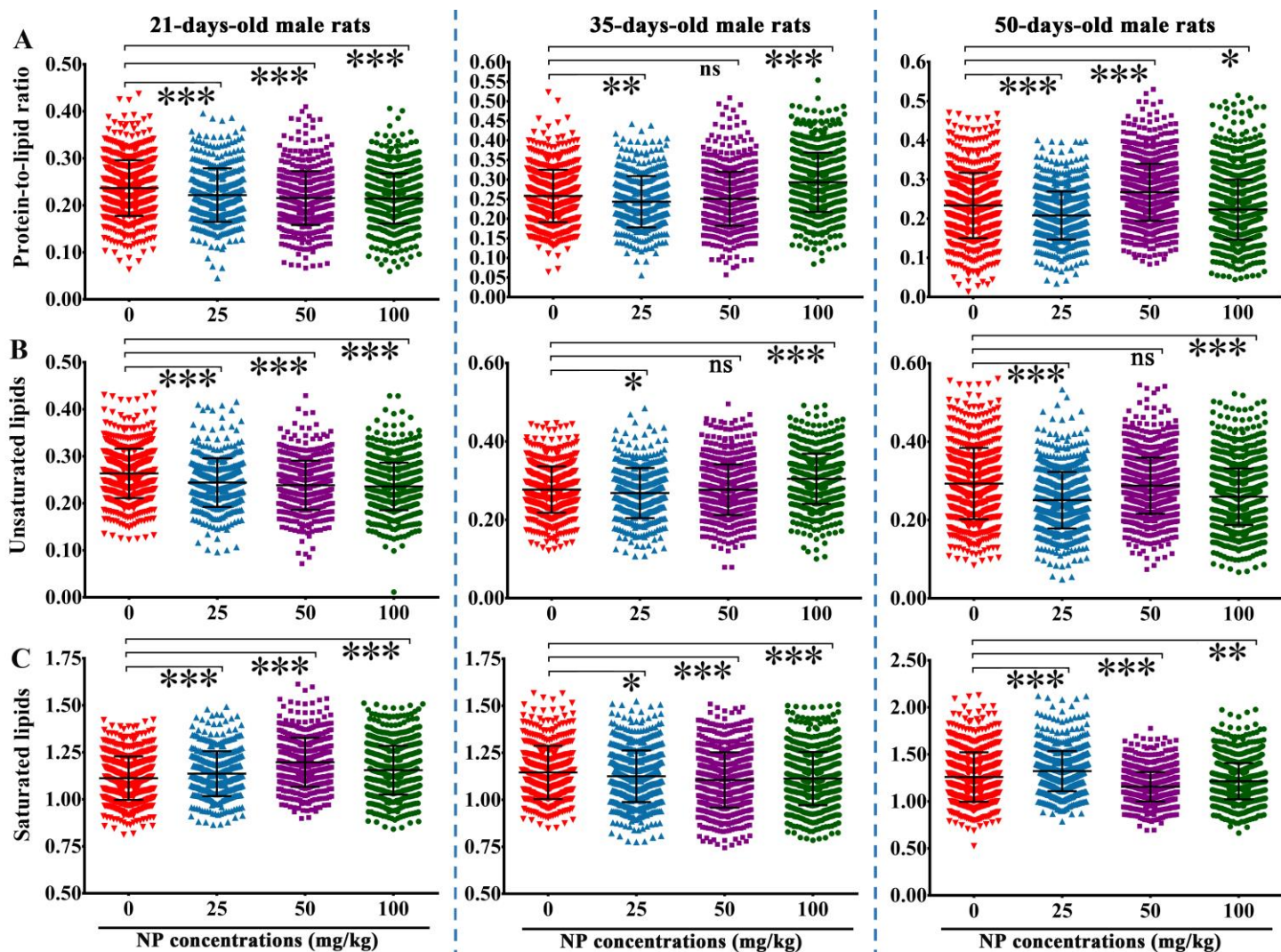
14 **Figure 2. Cluster vector plots comparing the control (red line at origin) and 4-**
 15 **Nonylphenol (NP)-treated groups. (A) 21-day-old rats; (B) 35-day-old rats; (C) 50-**
 16 **day-old rats. The spectra cut at 1800-900 cm^{-1} (left column), were baseline-corrected**
 17 **and normalized to the Amide I peak prior to PCA-LDA, and the spectra cut between**
 18 **3100 and 2800 cm^{-1} (right column), were baseline-corrected and vector-normalized.**
 19 **Plots were generated following PCA-LDA and show the top eight discriminating**
 20 **wavenumbers (cm^{-1}) responsible for the separation between NP exposure and control**
 21 **groups (0 mg/kg NP). Data represent the average of six rats per group.**



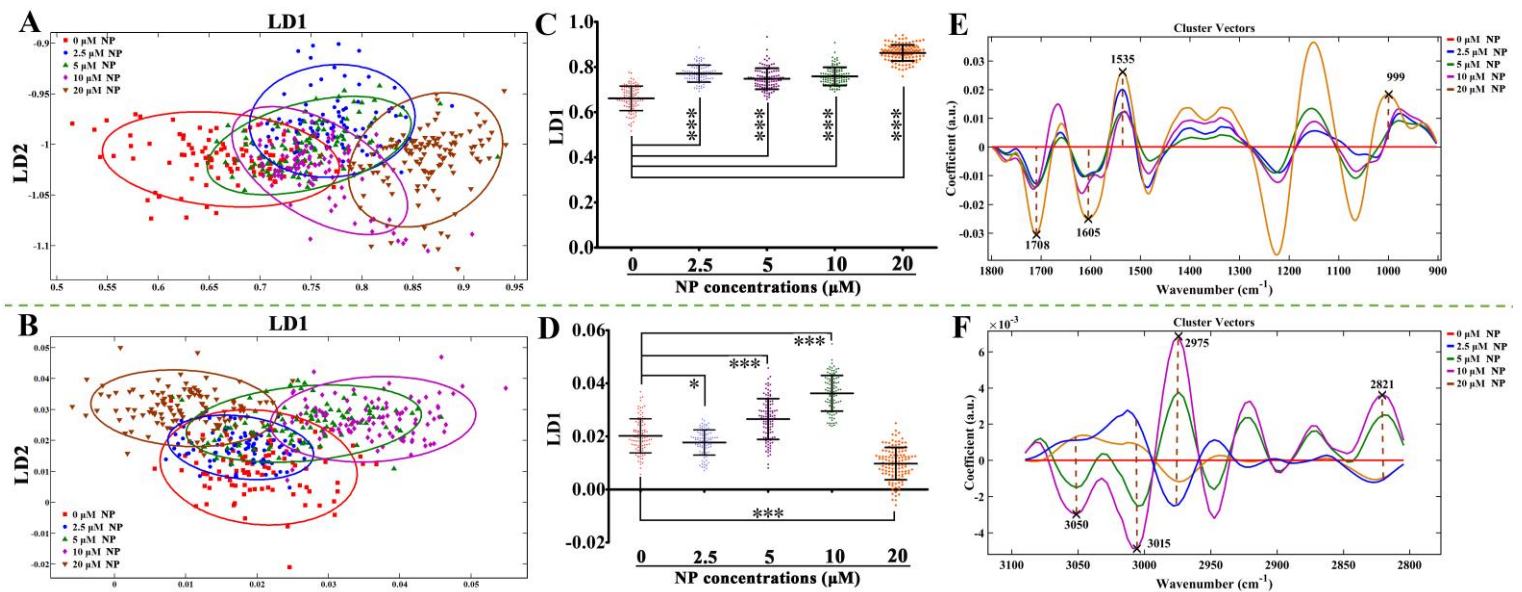
24 **Figure 3. Comparison of discriminating wavenumbers (cm^{-1}) with tentative**
 25 **biochemical assignments between control and 4-Nonylphenol (NP)-treated groups.**
 26 ATR-FTIR spectra were from the testicular cells of mice exposed to different
 27 concentrations of NP. **(A)** Lipid-to-protein ratio ($1740 \text{ cm}^{-1}/1400 \text{ cm}^{-1}$ ratio); **(B)**
 28 Peptide aggregation ($1630 \text{ cm}^{-1}/1650 \text{ cm}^{-1}$ ratio); **(C)** Amide I-to-Amide II ratio (1655
 29 $\text{cm}^{-1}/1545 \text{ cm}^{-1}$ ratio); **(D)** Phosphate-to-carbohydrate ratio [$(1055-1045) \text{ cm}^{-1}/(1555-$
 30 $1535) \text{ cm}^{-1}$ ratio]. All the data are represented as mean \pm standard deviations. $n=6$ for
 31 each group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus control group (0 mg/kg NP), one-
 32 way ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test.



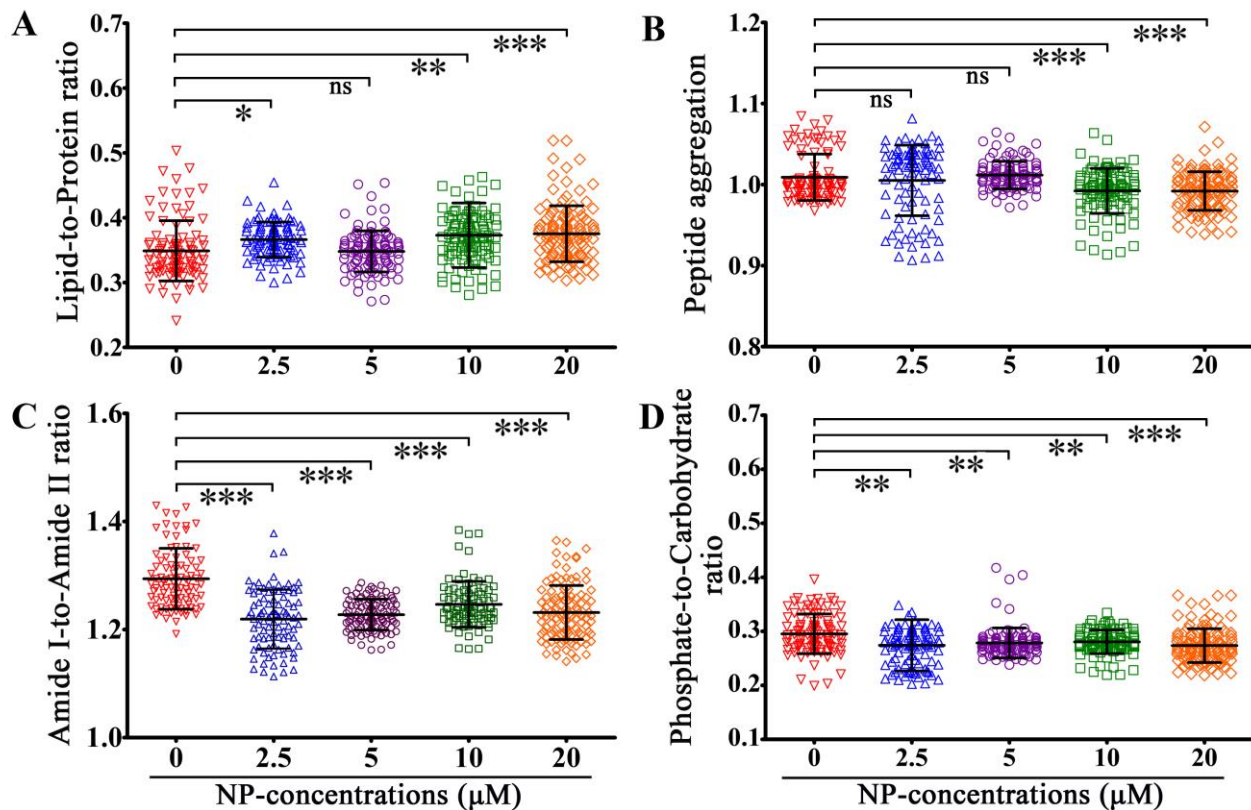
34 **Figure 4. PCA-LDA and resultant cluster vectors plots for Raman spectra**
 35 **extracted from testicular interstitial tissue in rats treated and untreated with 4-**
 36 **Nonylphenol (NP). (A) Top row: 21-day-old rats. (B) Middle row: 35-day-old rats. (C)**
 37 **Bottom row: 50-day-old rats. Three-dimensional (3-D) PCA-LDA scores plots (A, B**
 38 **and C), Linear discriminant 1 (LD1) scatter plots (D, E and F), cluster vectors plots (G,**
 39 **H and I), for Raman spectra region at 1800-900 cm⁻¹ (fingerprint region). Spectra were**
 40 **baseline-corrected and normalized to the Amide I peak. Confidence ellipsoids (90%)**
 41 **were drawn in each 3D scores plot. The data of each LD1 scatter plot is represented as**
 42 **mean ± standard deviations. Cluster vector plots were generated following PCA-LDA**
 43 **and show the top eight discriminating wavenumbers responsible for the separation**
 44 **between NP exposure and control groups. Data represent the average of six mice per**
 45 **group. Significance of category segregation was determined using one-way ANOVA**
 46 **with the Fisher's LSD or Dunnett's T3 post hoc test, ****P* < 0.001 versus control group**
 47 **(0 mg/kg NP).**



49 **Figure 5. Comparison of discriminating wavenumbers (cm^{-1}) with tentative**
50 **biochemical assignments between control and 4-Nonylphenol (NP)-treated groups.**
51 Raman spectra were from the testicular interstitial tissue of mice exposed to different
52 concentrations of NP. (A) Protein-to-lipid ratio ($1650\text{ cm}^{-1}/1440\text{ cm}^{-1}$ ratio); (B)
53 Unsaturated lipids ($1654\text{ cm}^{-1}/1445\text{ cm}^{-1}$ ratio); (C) Saturated lipids ($1303\text{ cm}^{-1}/1267$
54 cm^{-1} ratio). All the data are represented as mean \pm standard deviations. $n=6$ for each
55 group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus control group (0 mg/kg NP), one-way
56 ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test.



61 **Figure 6. PCA-LDA scores plots and resultant cluster vectors plots for ATR-FTIR**
62 **spectra acquired from Sertoli cells exposed to 4-Nonylphenol (NP) at various doses**
63 **(2.5, 5, 10 and 20 μM) compared to the control (0 μM NP). Upper row: two-**
64 **dimensional (2D) PCA-LDA scores plot of Linear discriminant 1 (LD1) versus Linear**
65 **discriminant 2 (LD2) (A), LD1 scatter plots (C) and cluster vectors plots (E) for ATR-**
66 **FTIR spectra region at 1800-900 cm⁻¹ with baseline-correction and normalization to the**
67 **Amide I peak (1650 cm⁻¹). Lower row: an expanded view (B, D and F) of the CH**
68 **stretching region 3100-2800 cm⁻¹, baseline-corrected and vector-normalized.**
69 **Confidence ellipsoids (90%) were drawn in each 2-D scores plot. The data of each LD1**
70 **scatter plot is represented as mean ± standard deviations of three experiments. Cluster**
71 **vectors plots were generated following PCA-LDA and show discriminating**
72 **wavenumbers. **P* < 0.05, ****P* < 0.001 versus control group (0 μM NP), one-way**
73 **ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test.**



75 **Figure 7. Comparison of discriminating wavenumbers (cm⁻¹) with tentative**
 76 **biochemical assignments between the control and 4-Nonylphenol (NP)-treated**
 77 **Sertoli cells.** ATR-FTIR spectra were from Sertoli cells treated with 0, 2.5, 5, 10 and
 78 20 μM NP for 12 h. **(A)** Lipid-to-protein ratio (1740 cm⁻¹/1400 cm⁻¹ ratio); **(B)** Peptide
 79 aggregation (1630 cm⁻¹/1650 cm⁻¹ ratio); **(C)** Amide I-to-Amide II ratio (1655 cm⁻¹
 80 ¹/1545 cm⁻¹ ratio); **(D)** Phosphate-to-carbohydrate ratio [(1055-1045) cm⁻¹/(1555-1535)
 81 cm⁻¹ ratio]. All the data are represented as mean ± standard deviations of three
 82 experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 *versus* control group (0 mg/kg NP), one-
 83 way ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test.