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Supplementary Material

1 Supplementary Figures

Supplementary Figure 1. Flow cytometry gating strategy
Purified pDCs and γδ T cells from healthy donors' blood were cocultured in absence or presence of TLR7L (CL097), TLR9L (CpG_A), phosphoantigens IPP or HMBPP together with zoledronate (Zol) or not. The phenotypic features of pDCs or γδ T cells were depicted by flow cytometry. (**A-C**) Upon pDC stimulation, γδ T cell features were defined: γδ T cells were defined using CD45, CD3, pan γδ TCR and δ2 markers (**A**), and CD69, CD25 and CD107 expression were further analyzed (**B**) together with immune checkpoints (LAG3 is shown) (**C**). (**D-F**) Upon γδ T cell activation, pDC features were depicted: pDCs were determined using CD45, HLA-DR and BDCA4 markers (**D**), and expression of CD40, CD86 and TRAIL was analyzed (**E**) together with immune checkpoints (PDL1 is shown) (**F**).

Supplementary Figure 2. pDCs trigger phenotypic modulation of γδ T-cell subsets
Purified pDCs and γδ T cells from healthy donors' blood were cocultured in absence (white dots) or presence of TLR7L (CL097) (light gray symbols), TLR9L (CpG_A) (dark gray symbols) together with zoledronate (Zol) (square symbols) or not (round symbols). The phenotypic features of γδ T cells including δ2+ and δ2- subsets were depicted by flow cytometry. (**A**) Expression of selected immune checkpoints and NKR by γδ T cells: GITR, OX40, NKp30, NKp44 and ICOS (n= 7 to 11). (**B-C**) Activation status of δ2+ and δ2- T-cell subsets evaluated by assessing CD69 (**B**) and CD25 (**C**) expression (n=13 to 26). (**D**) Expression of selected immune checkpoints and NKR by δ2+ and δ2- T-cell subsets: TIM3, LAG3, PD-1, 41BB, and NKG2D (n=7 to 11). (**E**) The cytotoxic activity of δ2+ and δ2- T-cell subsets was evaluated through CD107 surface expression upon subsequent coculture with melanoma tumor cells (n=7 to 21). P-values were calculated using the Wilcoxon matched pairs test with Bonferroni correction.

Supplementary Figure 3. TLR7/9L stimulation triggers pDC activation while Zol and HMB-PP have no direct impact on pDC' features
Purified pDCs from healthy donors' blood were cultured with or without (**A**) TLR7L (CL097), TLR9L (CpGA) or zoledronate (Zol) (n=9 to 11) or (**B**) HMB-PP alone or combined with Zol (n=4). The phenotypic and functional features of pDCs were then assessed through CD40, CD80 and CD86 expression, TRAIL exposure and IFNα secretion. Bars represents mean±SEM. P-values were calculated using the Wilcoxon matched pairs test with Bonferroni correction.

Supplementary Figure 4. HMB-PP elicits activation and functionality of γδ T cells while TLR7/9L and Zol have no direct impact on γδ T cells' features
Purified γδ T cells from healthy donor' blood were cultured with or without (**A**) HMB-PP (n=13 to 25) or (**B,C**) TLR7L (CL097), TLR9L (CpGA), zoledronate (Zol) alone or in combination (n=4). The phenotypic and functional features of γδ T cells, δ2+ and δ2- T-cell subsets were assessed through CD69, CD25 expression, IFNγ secretion and CD107 exposure upon further coculture with tumor cells. Bars represents mean±SEM. P-values calculated using the Wilcoxon matched pairs test with Bonferroni correction.

Supplementary Figure 5. The interplay between pDCs and γδ T cells require the activation of one partner

(**A-B**) Purified pDCs or γδ T cells from healthy donors' blood were cultured alone (white symbols) or in presence of the cross-talk partner (dark symbols) in absence of any stimulation. (**A**) The phenotypic and functional features of γδ T cells were assessed through CD69 and CD25 expression, IFNγ secretion and CD107 exposure upon further coculture with tumor cells (n=18 to 26). Bars represents mean±SEM. (**B**) The phenotypic and functional features of pDCs were assessed through CD40, CD80 and CD86 expression, TRAIL exposure and IFNα secretion (n=9 to 12). (**C**) IFNγ secretion during γδ T cell/pDC co-culture in absence of stimulation (-) or in presence of HMB-PP or Zol. Bars represents mean±SEM. P-values were calculated using the Wilcoxon matched pairs test.

Supplementary Figure 6. Membrane contacts and soluble factors are required for effective cross-talk between pDCs and δ2+ / δ2- T-cell subsets

Purified pDCs and γδ T cells from healthy donor' blood were cocultured together in the same well (white bars) or physically separated by a 0.4μm membrane (transwell, gray bars) in absence or presence of TLR7L (CL097), TLR9L (CpG_A) or zoledronate (Zol) as indicated. The phenotypic features (CD69 and CD25 expression) and cytotoxic properties (CD107 exposure upon coculture with tumor cells) of δ2+ and δ2- T-cell subsets were assessed to identify the requirement for membrane contacts and/or soluble factors for their cross-talk with pDCs (n=8). Bars represents mean±SEM. P-values were calculated using the Wilcoxon matched pairs test.

Supplementary Figure 7. Experimental schemes for blocking experiments

(A) Strategy for blocking single molecules, BTN3A or mixtures to determine the mechanism of the pDC- γδ T cells cross-talk. (**B**) Strategy for blocking single molecules, BTN3A or mixtures to determine the mechanism of the γδ T cell-pDC cross-talk.

Supplementary Figure 8. pDCs and γδ T cells differentially expressed TNFαRI, TNFαRII and IFNαRI/RII

(A) Basal expression of TNFαRI, TNFαRII, IFNαRI and IFNαRII by γδ T cells and pDCs determined within PBMC (n=4). (**B**) The expression of TNFαRI, TNFαRII, IFNαRI and IFNαRII by γδ T cells and pDCs was evaluated during the coculture of purified pDCs and γδ T cells in presence or not of HMB-PP, CLO97, CpGA or Zol (n=2).

Supplementary Figure 9. The blocking of BTN3A or the use of the Supermix doesn't prevent pDC response to TLR7/9L stimulation

Purified pDCs from healthy donor' blood were cultured with or without TLR7L (CL097), TLR9L (CpG_A) or zoledronate (Zol) in presence or not of blocking antibodies. The ability of pDCs to upregulate activation molecules (CD40, CD80, CD86), express TRAIL and secrete IFNα was then evaluated. (**A**) Cultures performed in presence or not of anti-BTN3A blocking antibody (n=6). (**B**) Cultures performed in presence or not of the Supermix composed of anti-IFNAR, -TNFR1, -TNFR2 and -OX40 blocking antibodies together with anti-BTN3A1 blocking antibodies (n=6). Bars represents mean±SEM. P-values were calculated using the Wilcoxon matched pairs test.

Supplementary Figure 10. Relative impact of BTN3A compared to the other molecules targeted by the Supermix on the pDCs-γδ T cells cross talk

Purified pDCs from healthy donors' blood were pre-incubated either with single anti-BTN3A antibodies (n=12) or a Supermix composed of anti-IFNAR2, -TNFR1/TNFR2 and -OX40 antibodies together with anti-BTN3A blocking antibody (n=12), and cocultured with purified γδT cells in the presence or not of TLR7-L (CL097) (light gray bars), TLR9-L (CpG_A) (dark gray bars) or zoledronate (Zol) (black bars). The features of γδT cells were then depicted: the activation status (CD25 and CD69 expression) and the cytotoxic activity (CD107 surface exposure) were analyzed by flow cytometry; IFNγ secretion was measured by CBA in the supernatants. Bars represents mean±SEM. P-values were calculated using the Wilcoxon-matched pairs test (full lines) or Mann-Whitney test (dotted lines) (*p<0.05,**p<0.01,***p<0.001).

Supplementary Figure 11. γδ T cells keep their ability to respond to HMB-PP stimulation in presence of blocking antibodies composing the "mix -" or blocking of BTN3A

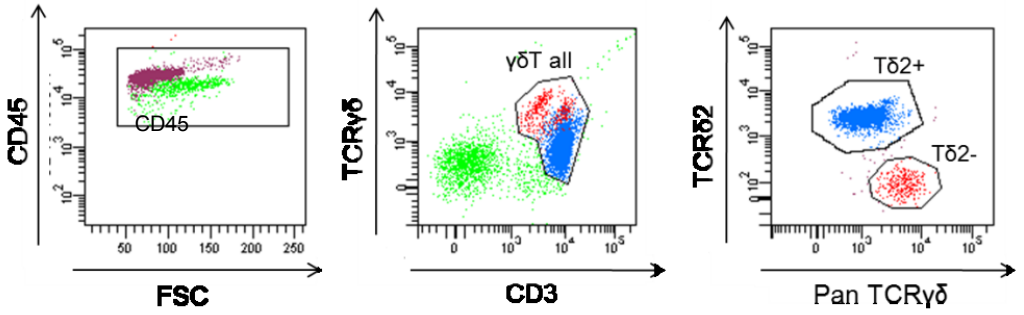
(A) Purified γδ T cells from healthy donor' blood were cultured with or without HMB-PP or zoledronate (Zol) in presence or not of the "mix -" composed of anti-IFNγR, -NKp30 and -GITR blocking antibodies (n=4-10). The ability to upregulate activation molecules (CD69, CD25) and secrete IFNγ was then evaluated on γδ T cells. (**B**) Purified γδ T cells from healthy donor' blood were pre-activated for 4 hours with or without HMB-PP or zoledronate (Zol) and mixed with purified pDCs pre-cultured for 2 hours in presence or not of anti-BTN3A blocking antibodies (n=8). The ability to upregulate activation molecules (CD69, CD25) and secrete IFNγ was then evaluated on γδ T cells. P-values were calculated using the Wilcoxon matched pairs test.

Supplementary Figure 12. Relative impact of BTN3A compared to the other molecules targeted by the Supermix on the γδ T cells-pDCs cross talk

Purified γδT cells from healthy donor' blood were pre-activated with HMB-PP (light gray symbols) or Zol (dark gray symbols) during 4hours in absence (condition "BTN3A", n=8) or presence of a mixture of blocking antibodies composed of anti-IFNγR, -NKp30, and -GITR antibodies (condition "Supermix", n=9-10), and mixed with pDCs pre-blocked with single anti-BTN3A for 2hours. The features of pDCs were then depicted: the activation status (MFI CD40 on CD40-positive cells) and cytotoxic properties (TRAIL) were analyzed by flow cytometry, whereas the cytokine secretion (IFNα and IP10) was assessed by CBA in the supernatants. P-values were calculated using the Wilcoxon-matched pairs test (full lines) or Mann-Whitney test (dotted lines) (*p<0.05,**p<0.01,***p<0.001).

Supplementary Figure 1

A



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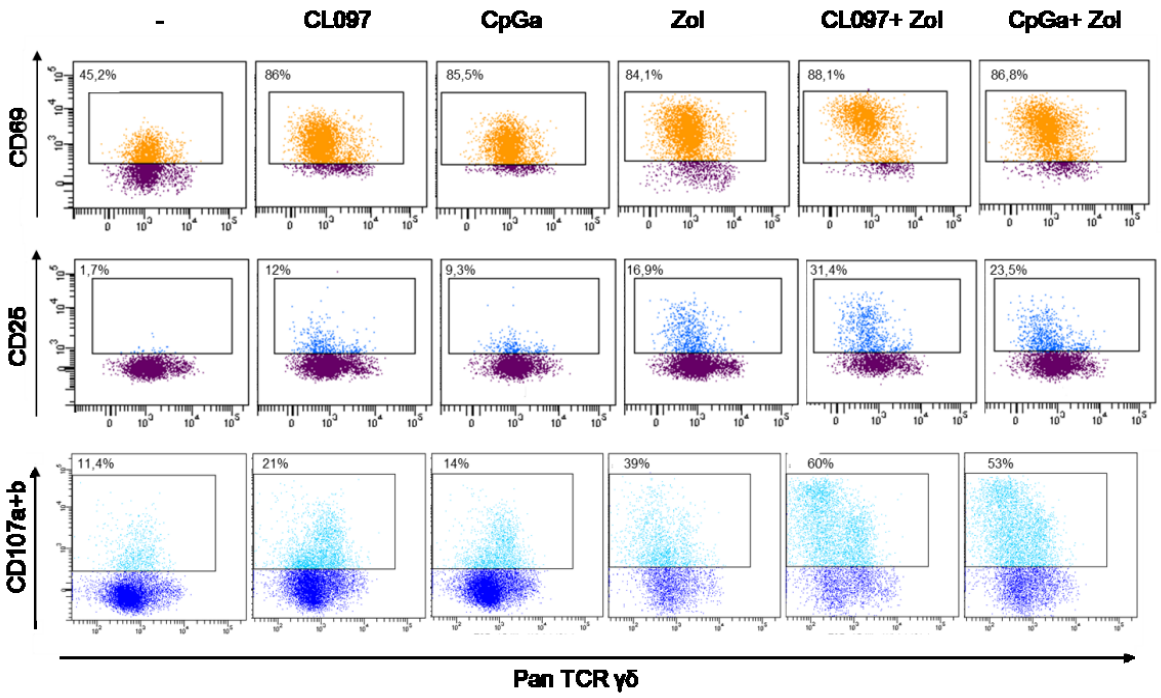

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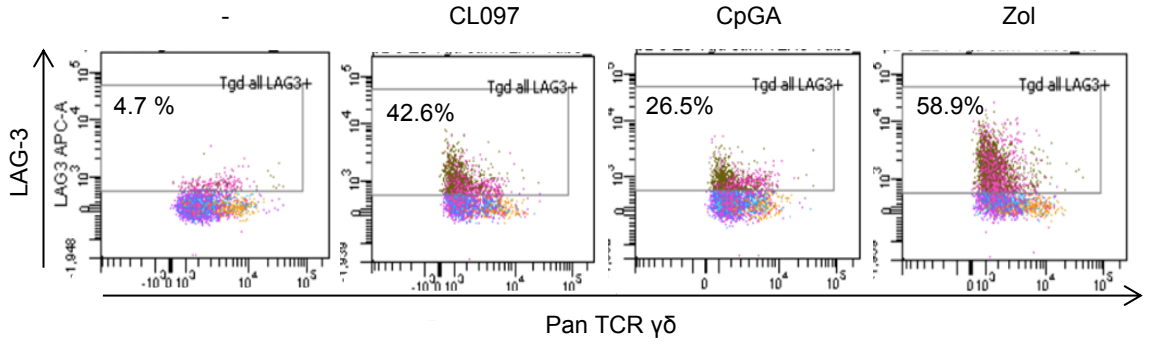

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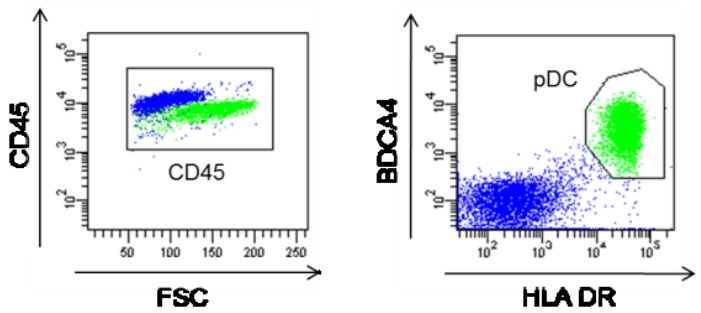

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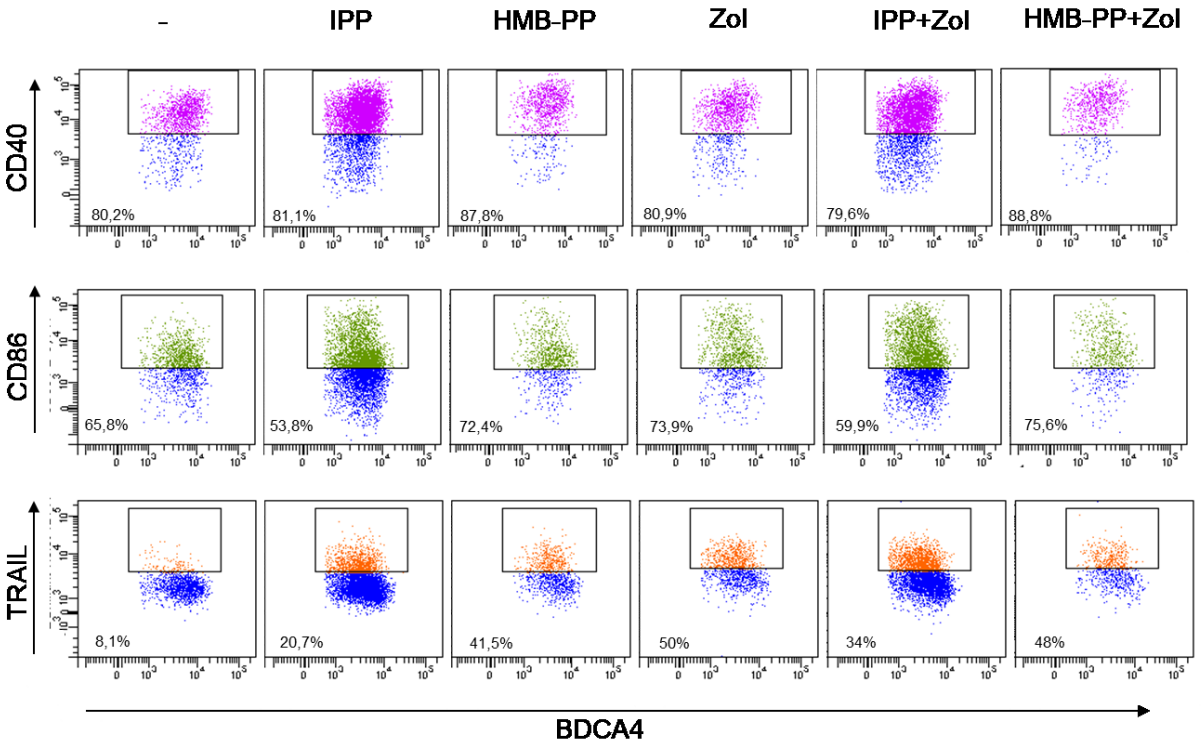

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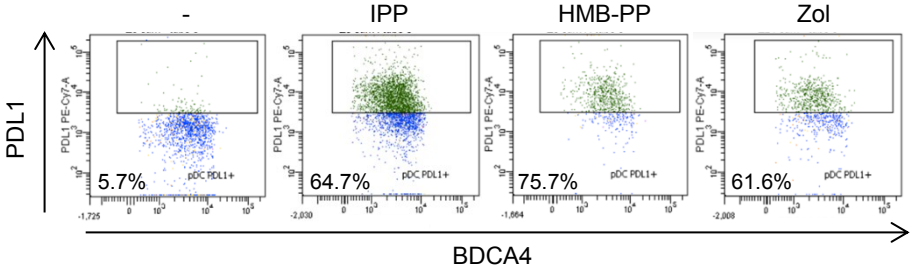

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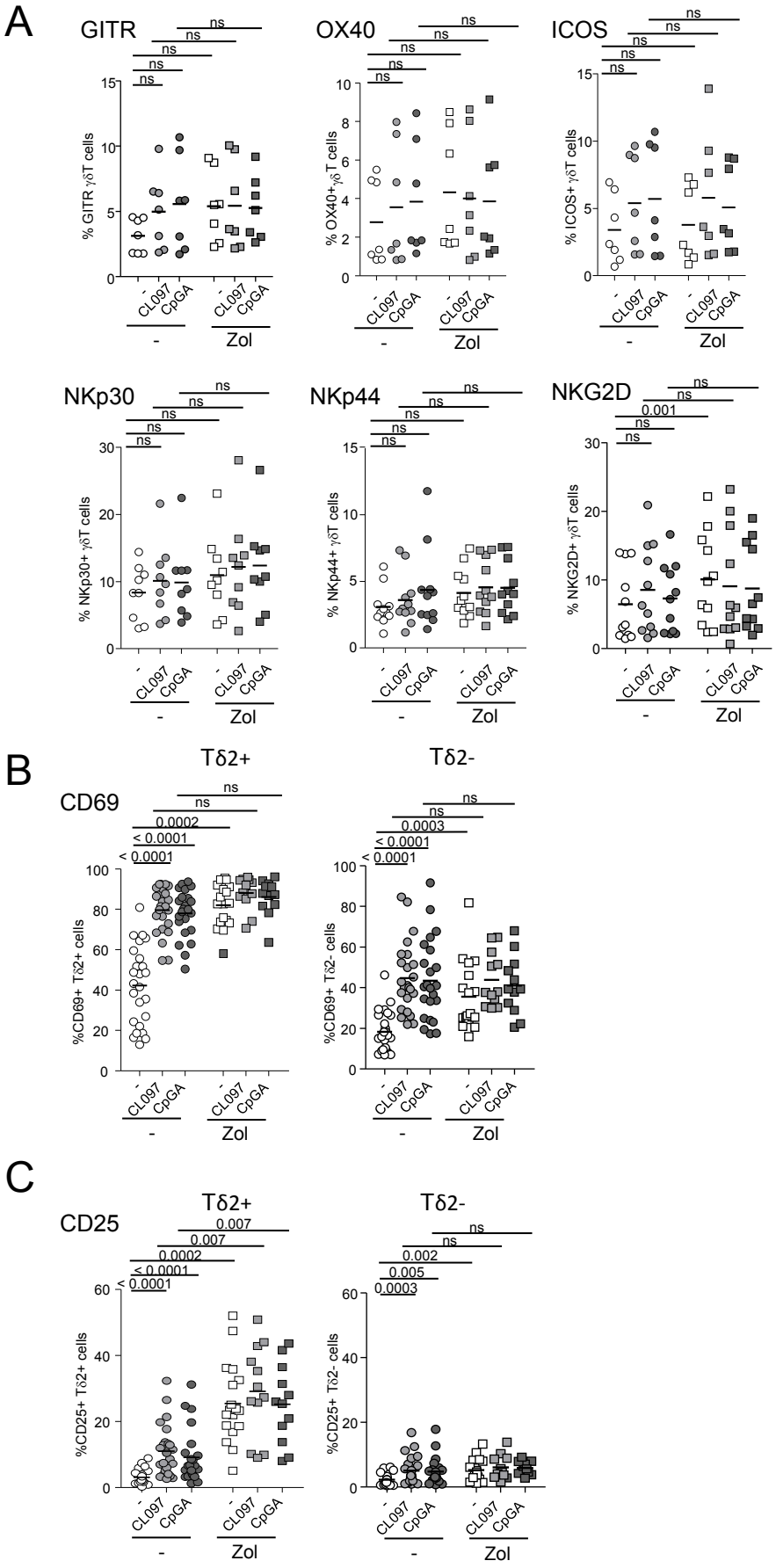

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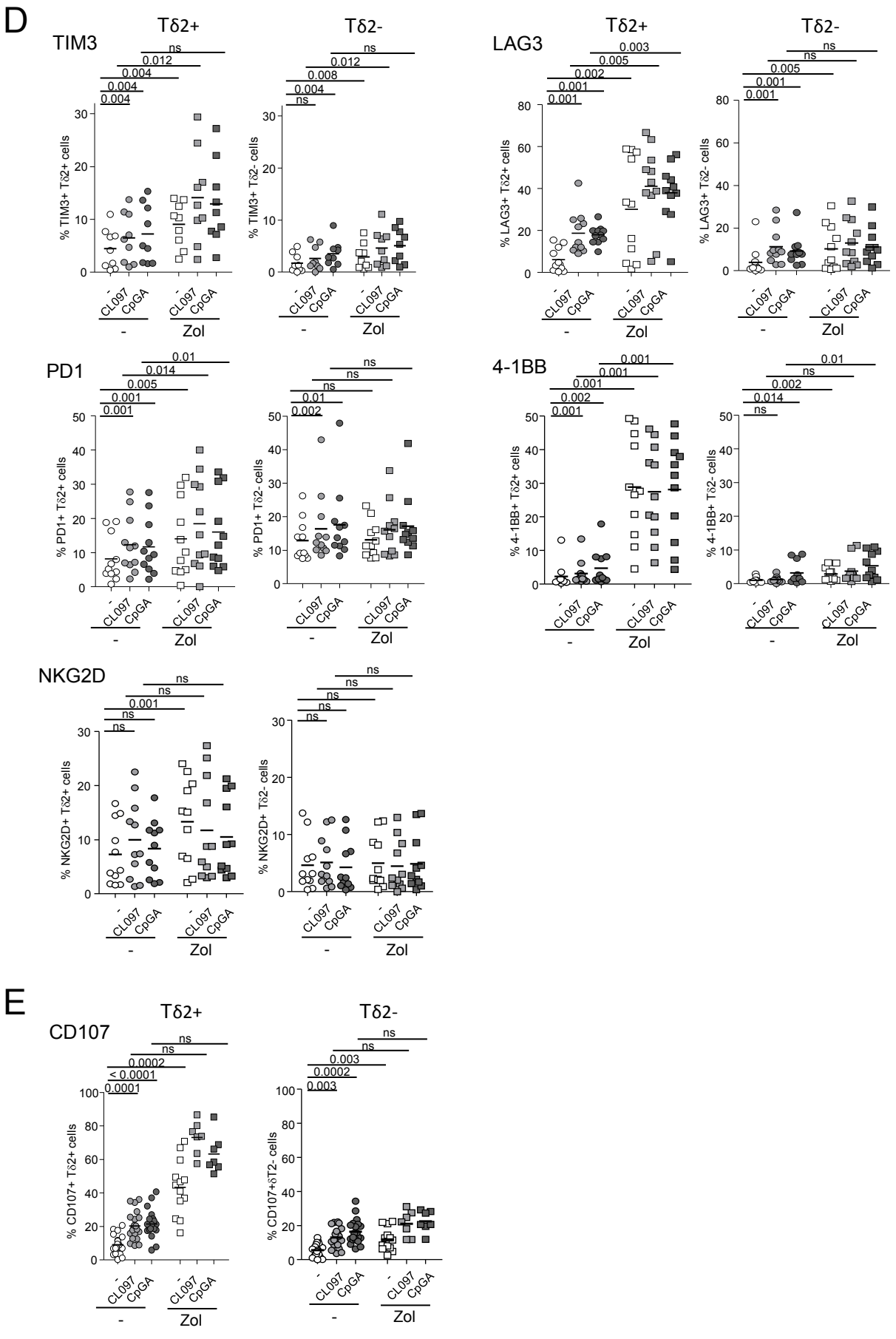

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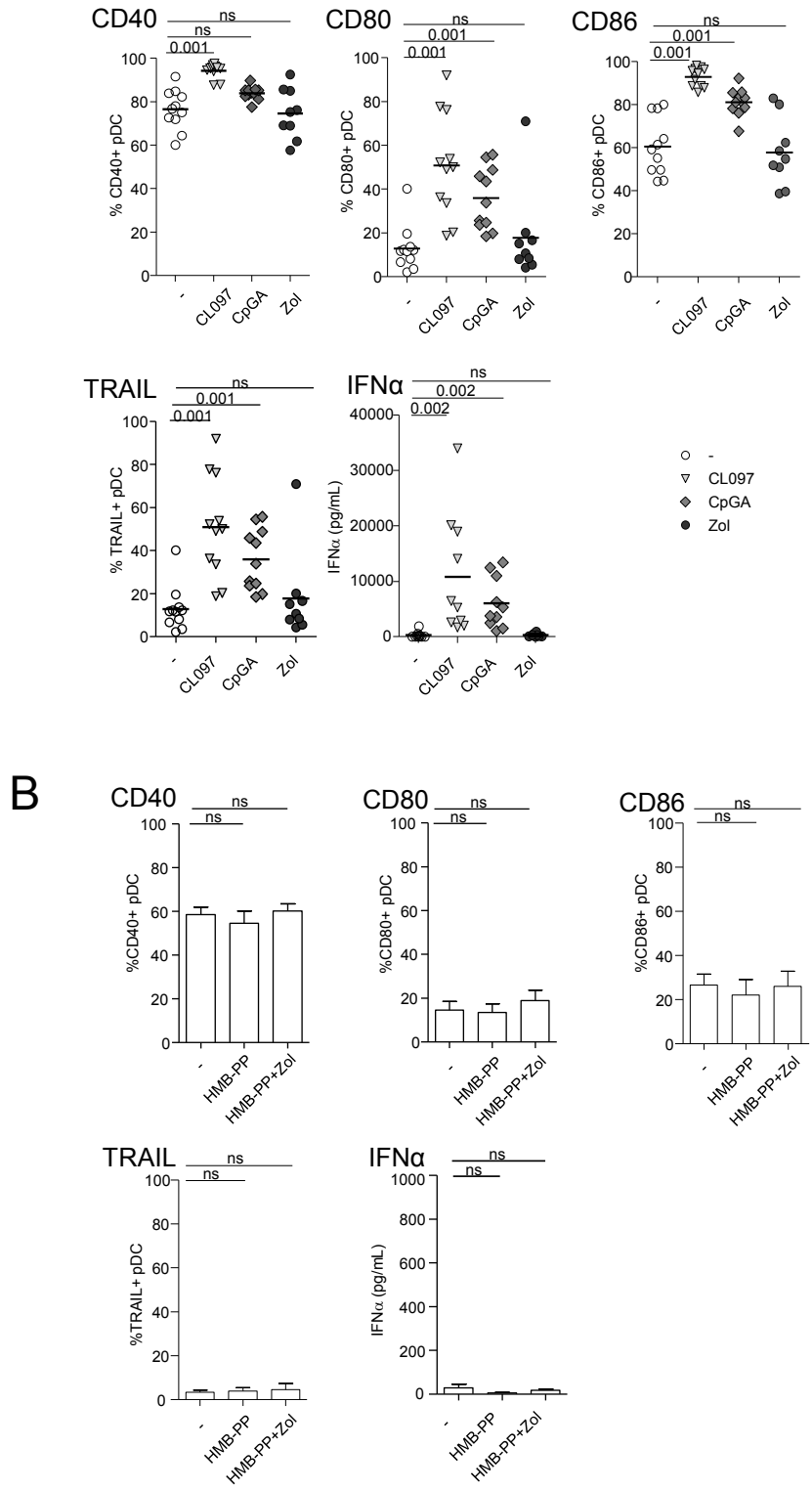

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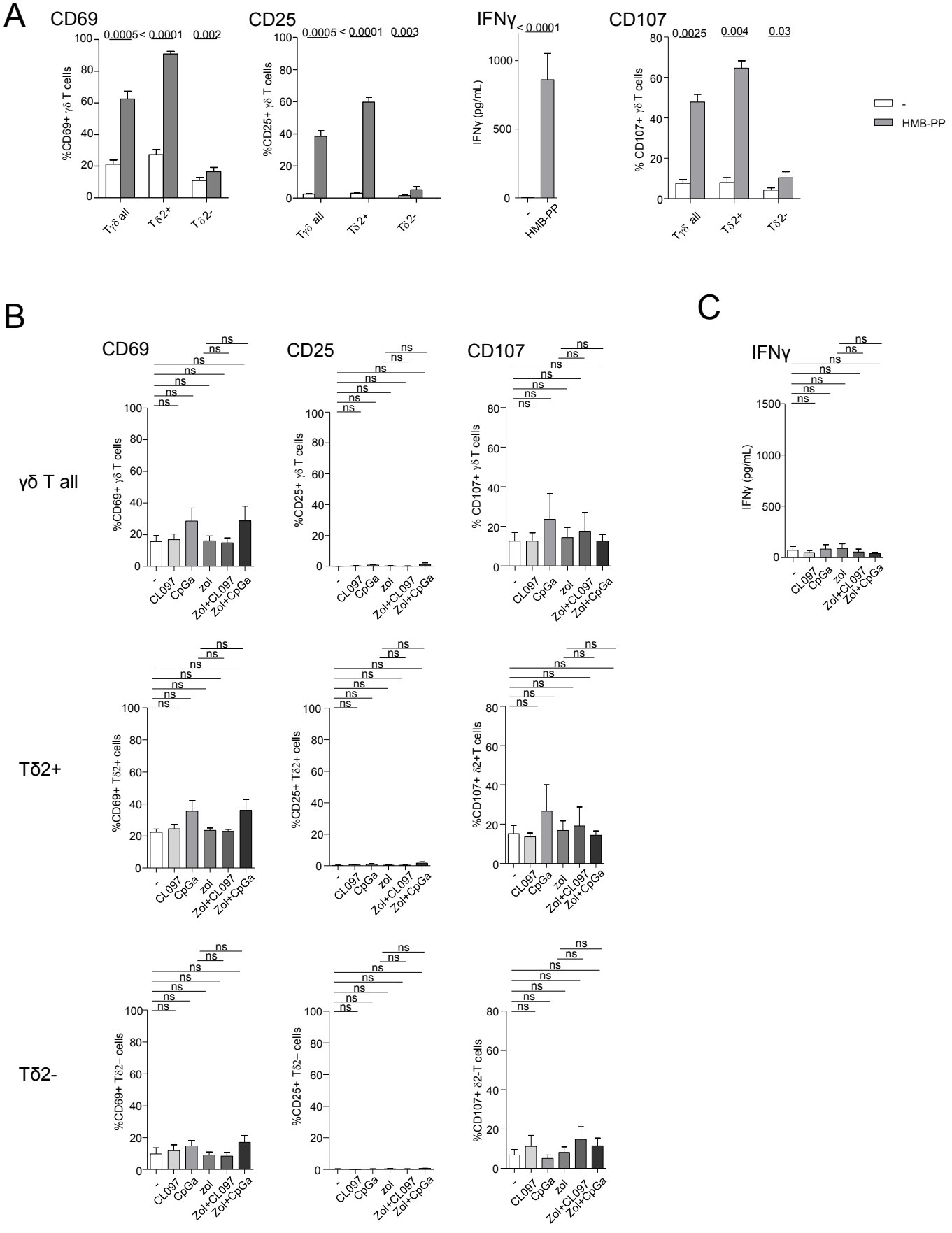

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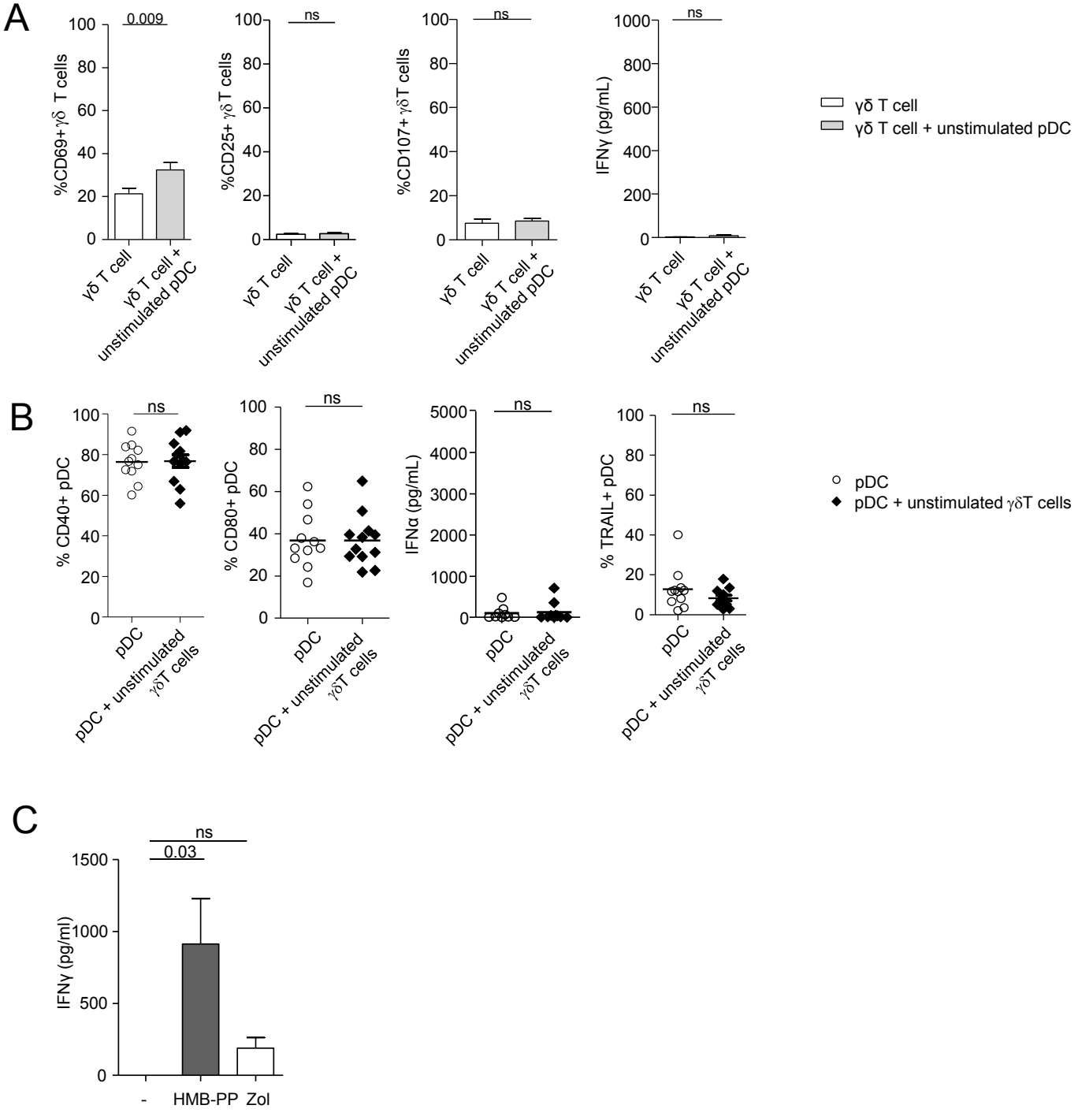

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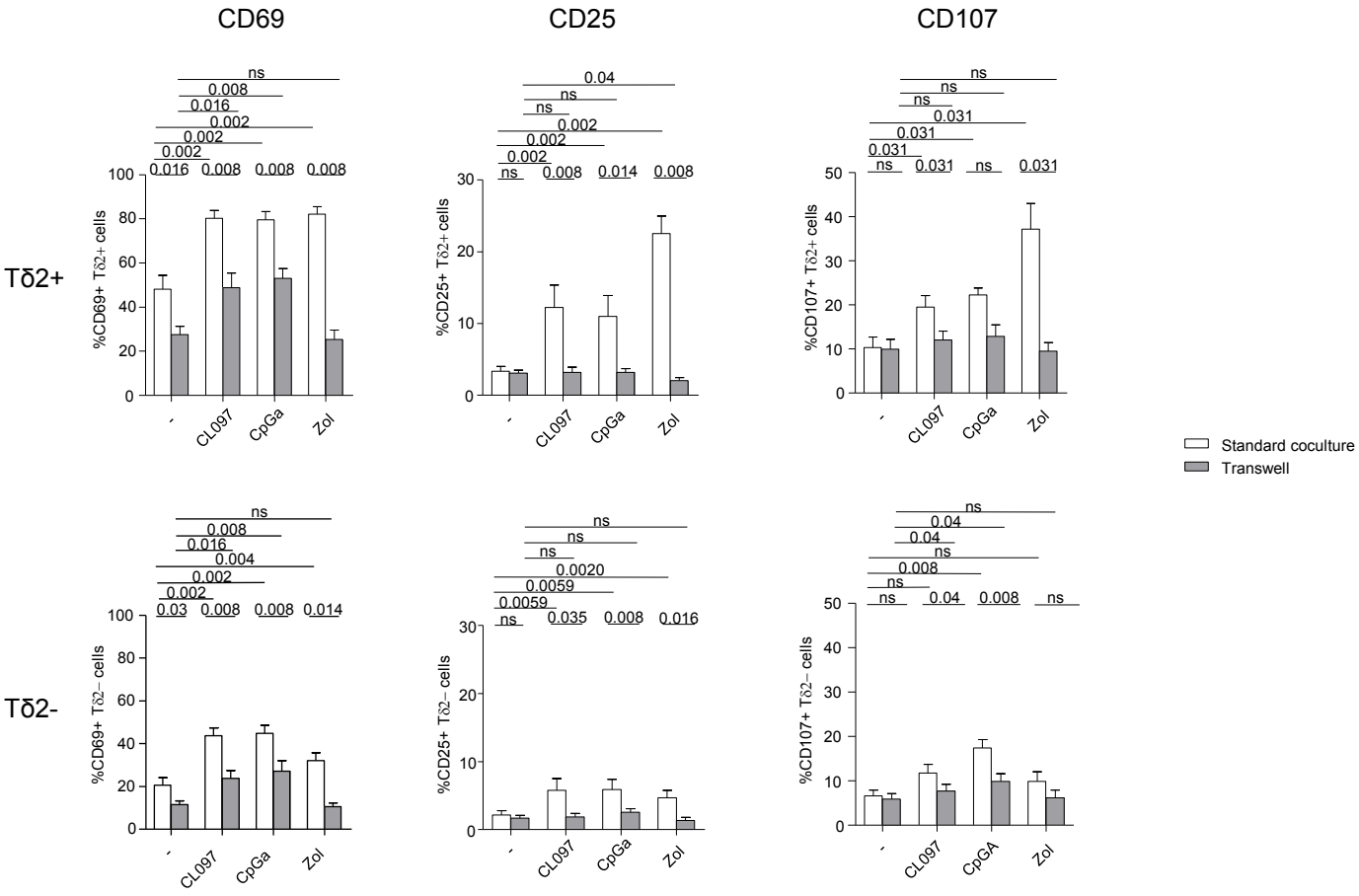

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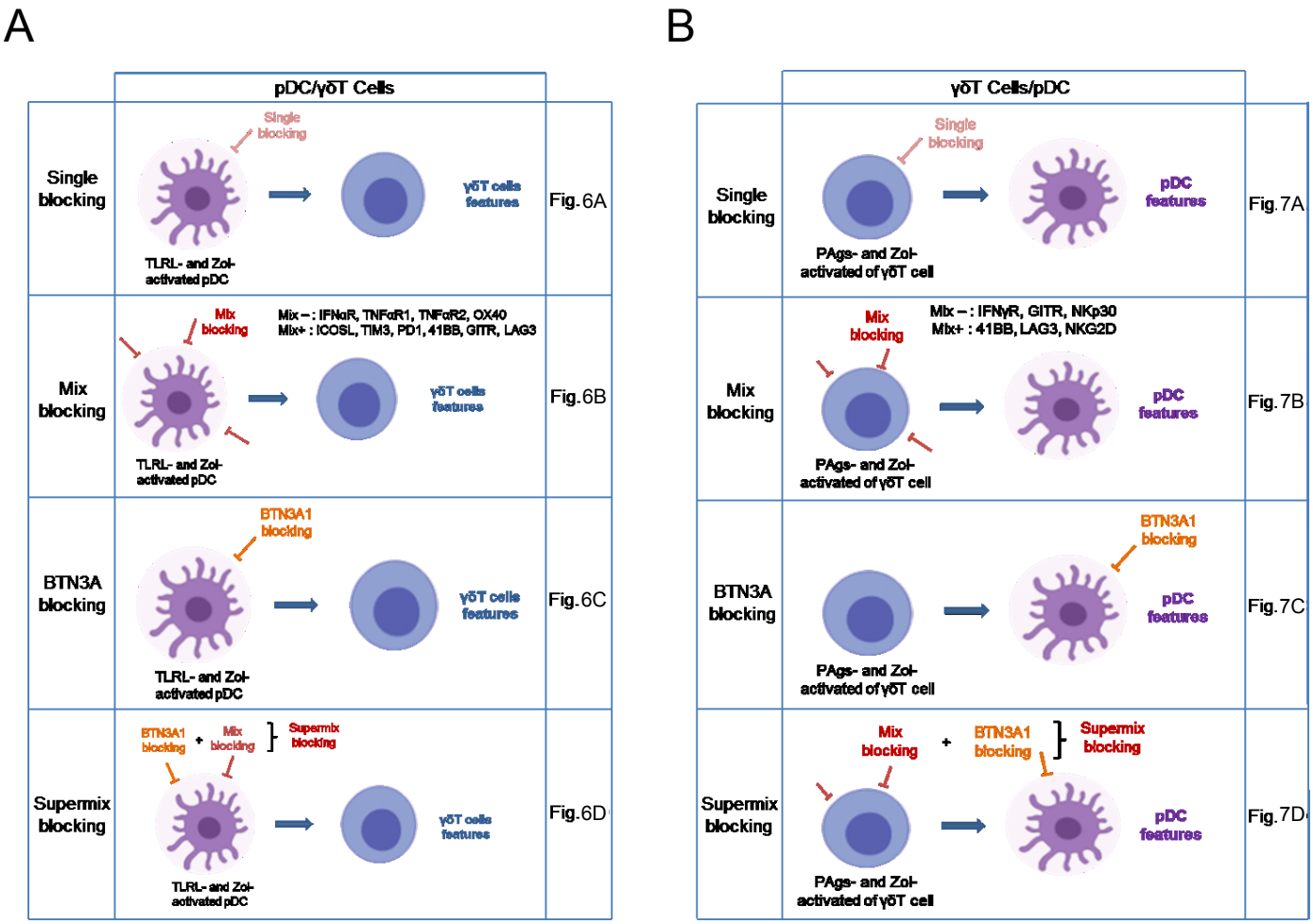

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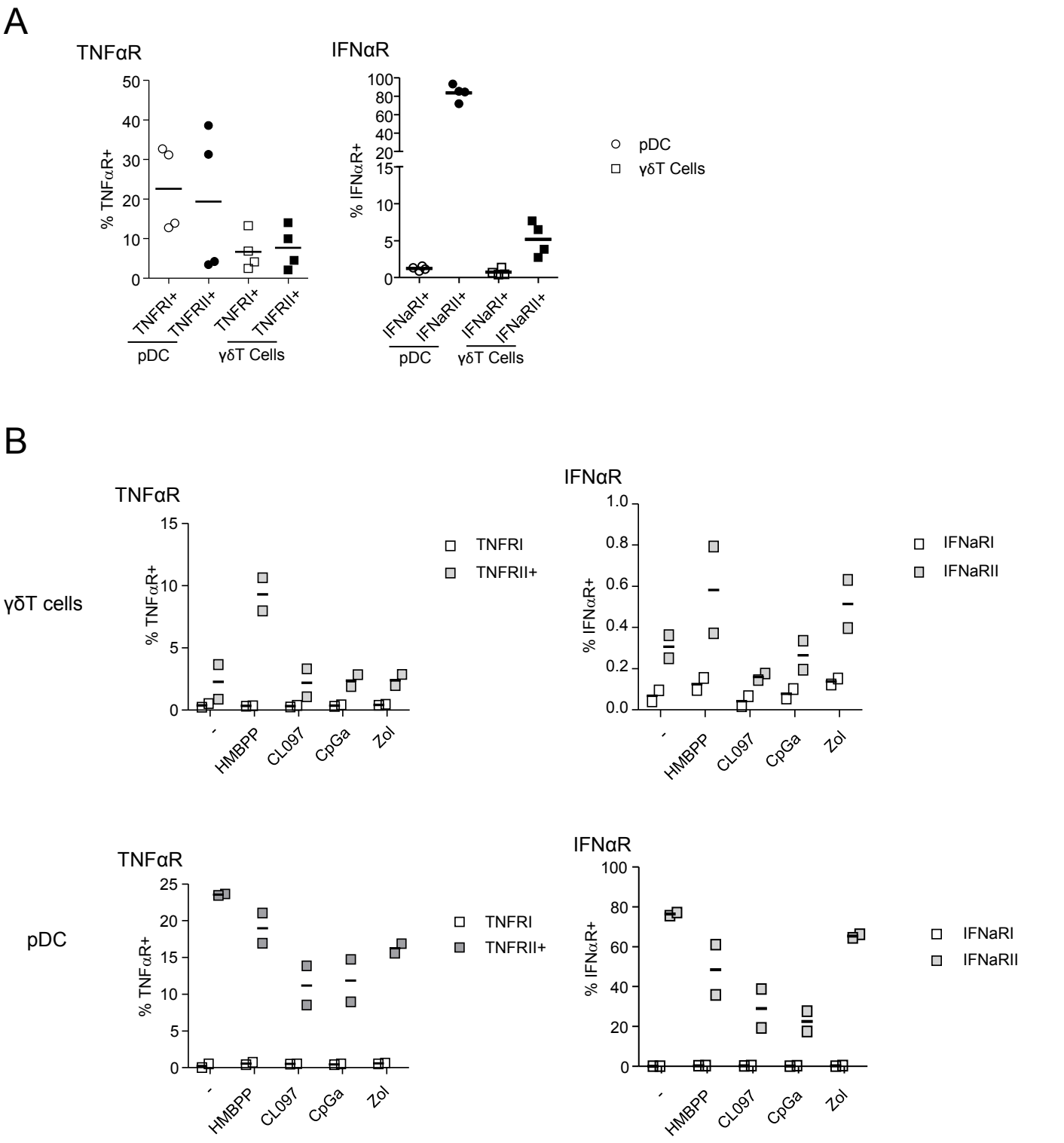

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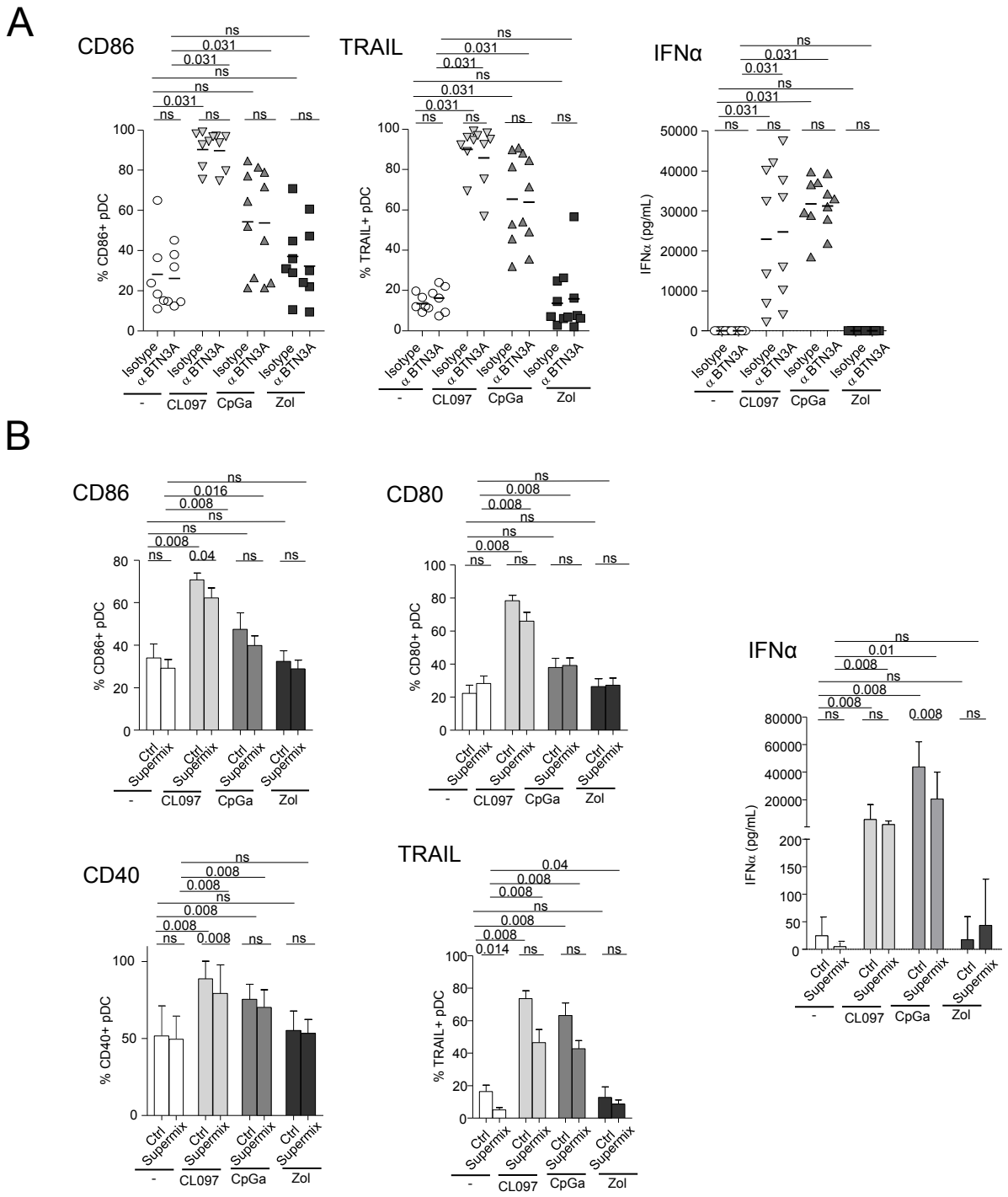

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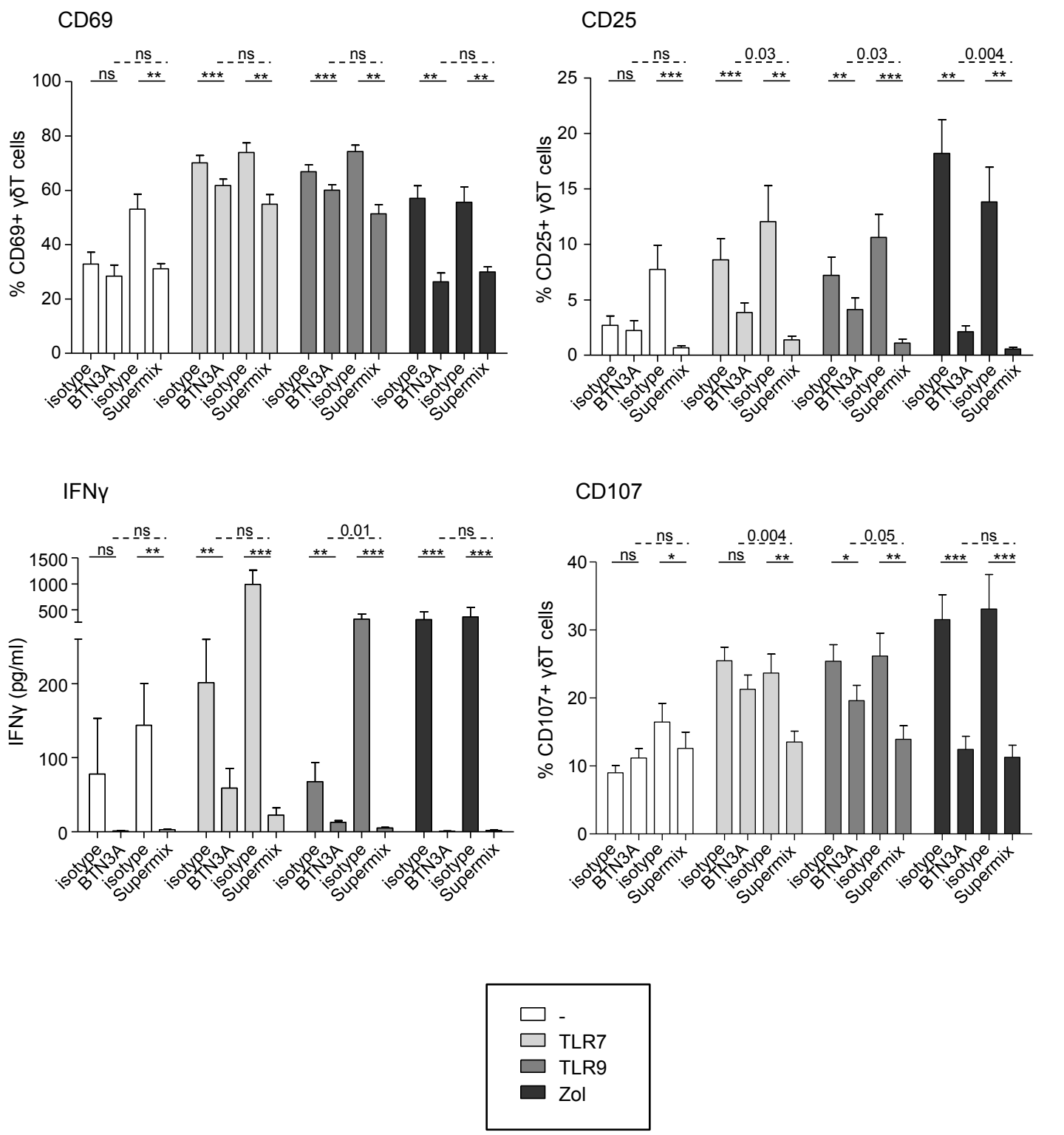

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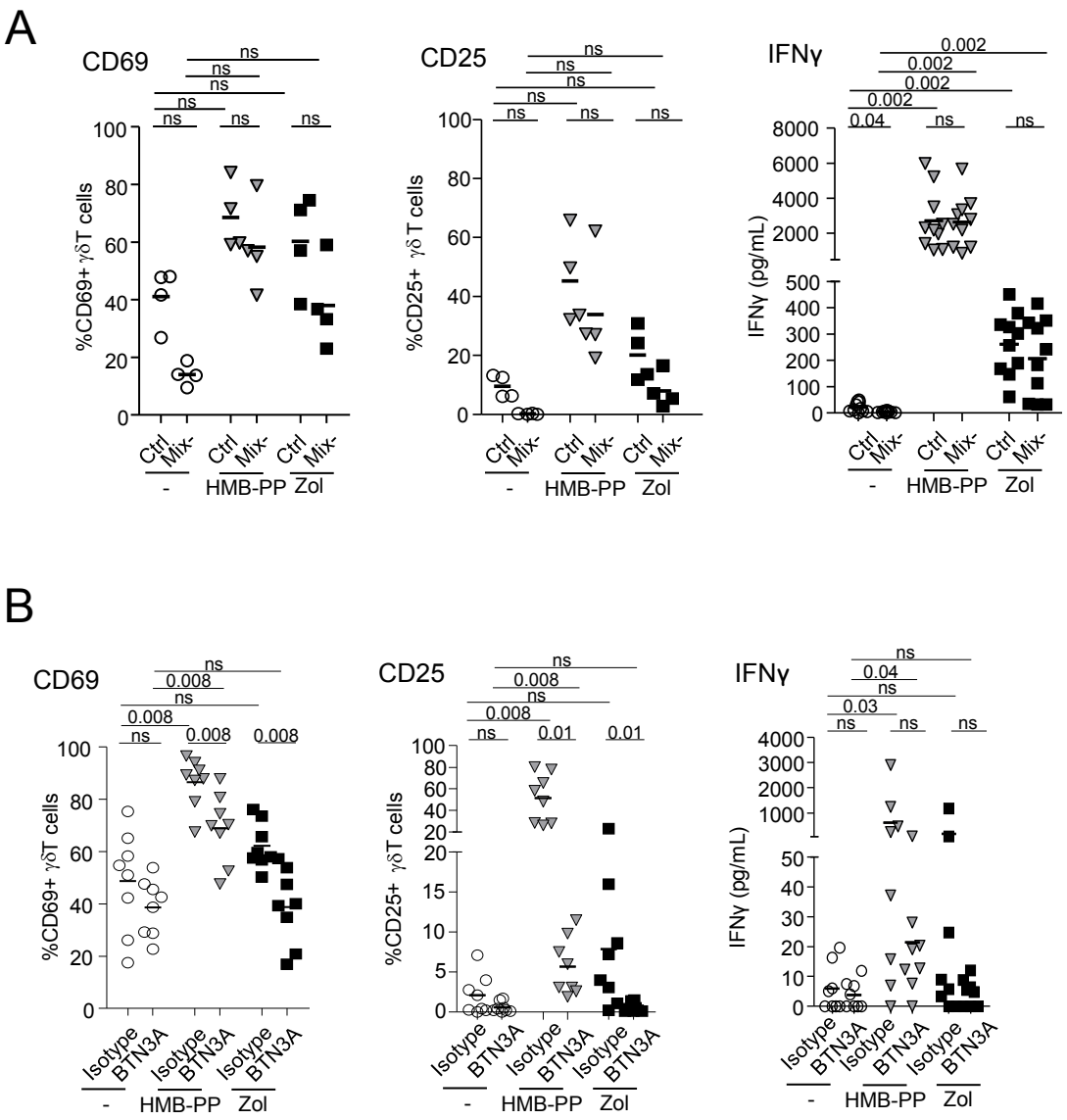

Supplementary Figure 12