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Supplemental information

B cell focused transient immune suppression protocol for efficient AAV readministration to the liver

Jyoti Rana, Roland W. Herzog, Maite Muñoz-Melero, Kentaro Yamada, Sandeep R.P. Kumar, Anh K. Lam, David M. Markusic, Dongsheng Duan, Cox Terhorst, Barry J. Byrne, Manuela Corti, and Moanaro Biswas

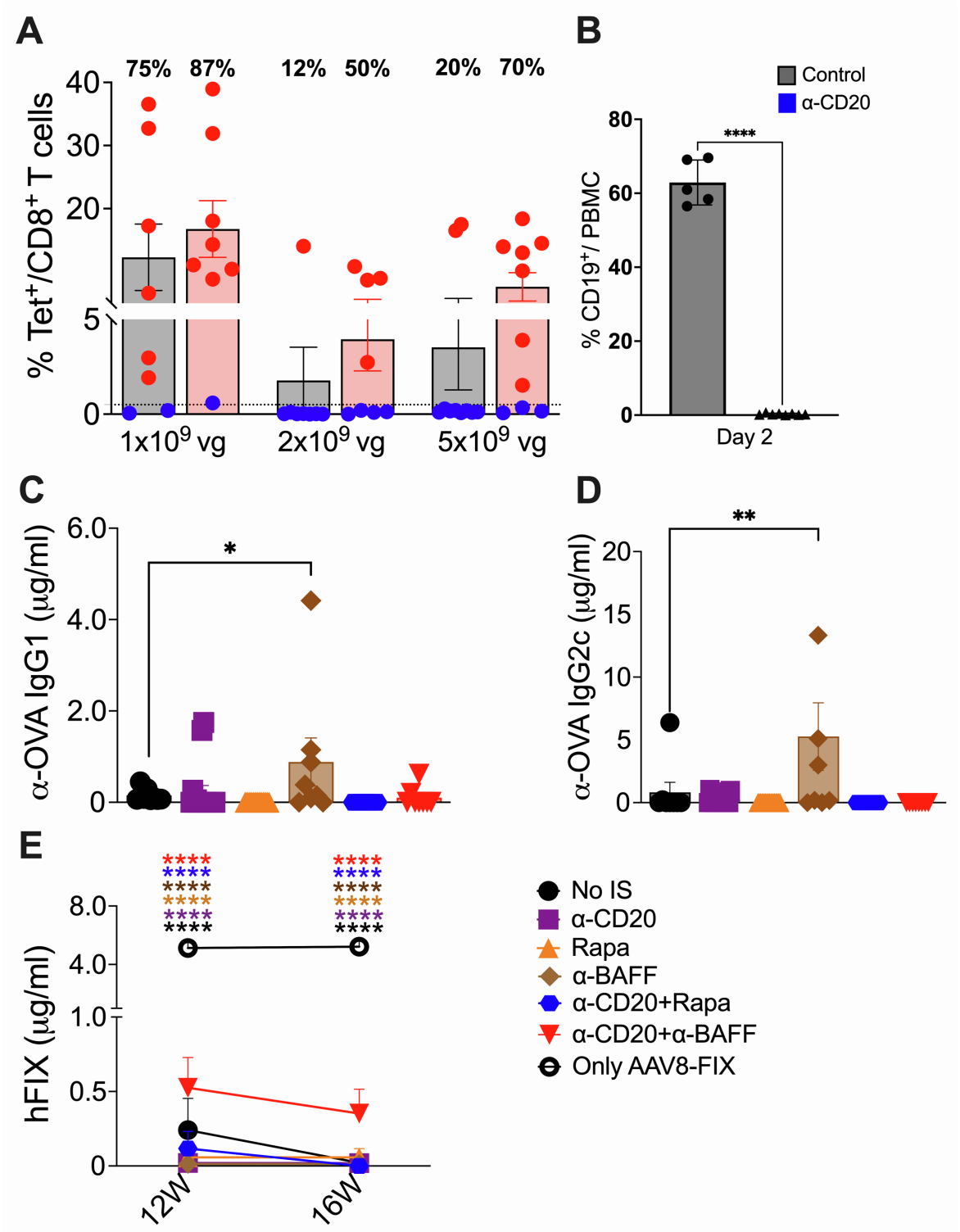

Figure S1: Effect of concurrent immune suppressive (IS) treatment. A) Comparison of $CD8^+$ T cell responses to AAV8-OVA administered at $1x10^9$, $2x10^9$, and $5x10^9$ vg/animal for no IS vs concurrent α-CD20 + α-BAFF treatment (n=8/group). Red dots indicate tetramer$^+$ (Tet^+) animals and blue dots indicate (Tet^-) animals. Frequency of Tet^+ animals in each group is indicated above each bar. **B)** Frequency of circulating B cells on day 2 after initiation of α-CD20 treatment. **C and D)** Comparison of OVA specific IgG1 and IgG2c levels from plasma samples collected at 4 weeks post AAV8-OVA and IS administration (n=5-8/group). **E)** Circulating hFIX levels at 12- and 16- weeks post initial dosing. Statistical significance was calculated by one-way ANOVA (Dunnett's multiple comparisons) for **B, C**, and **D**, two-way ANOVA for **E**. *p ≤ 0.05; **p ≤ 0.01; ****p ≤ 0.0001.

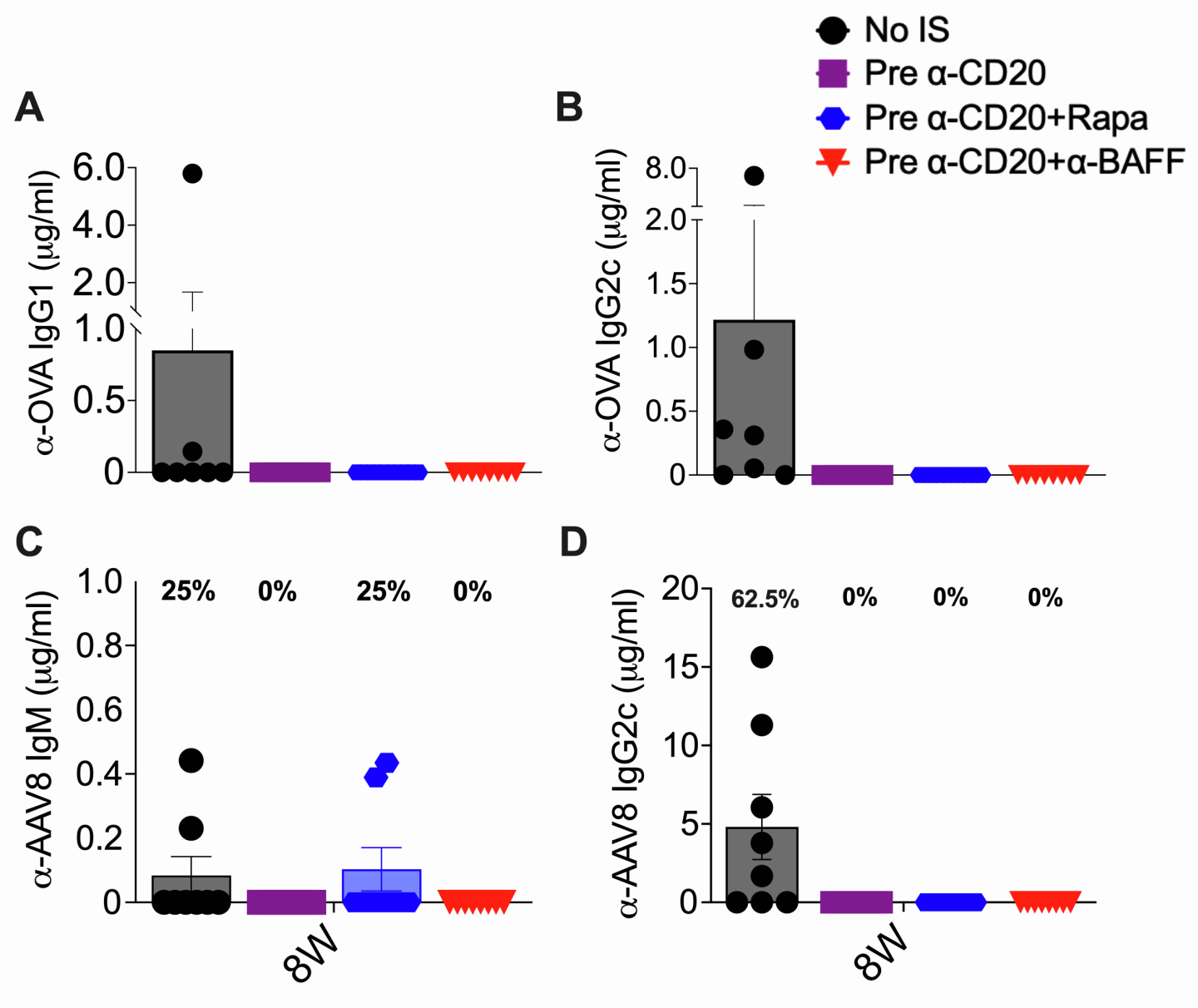

Figure S2: Effect of IS pre-treatment on primary low dose AAV administration. A and B) OVA specific IgG1 and IgG2c levels in plasma samples collected at 4 weeks post AAV8-OVA administration and IS pre-treatment (n=7-8/group). **C and D)** Frequencies of animals that develop AAV capsid specific IgM and IgG2c responses from plasma samples collected at 8 weeks post primary low dose AAV8-OVA administration. Statistical significance was calculated by one-way ANOVA (Dunnett's multiple comparisons) for **A,** and **B**.

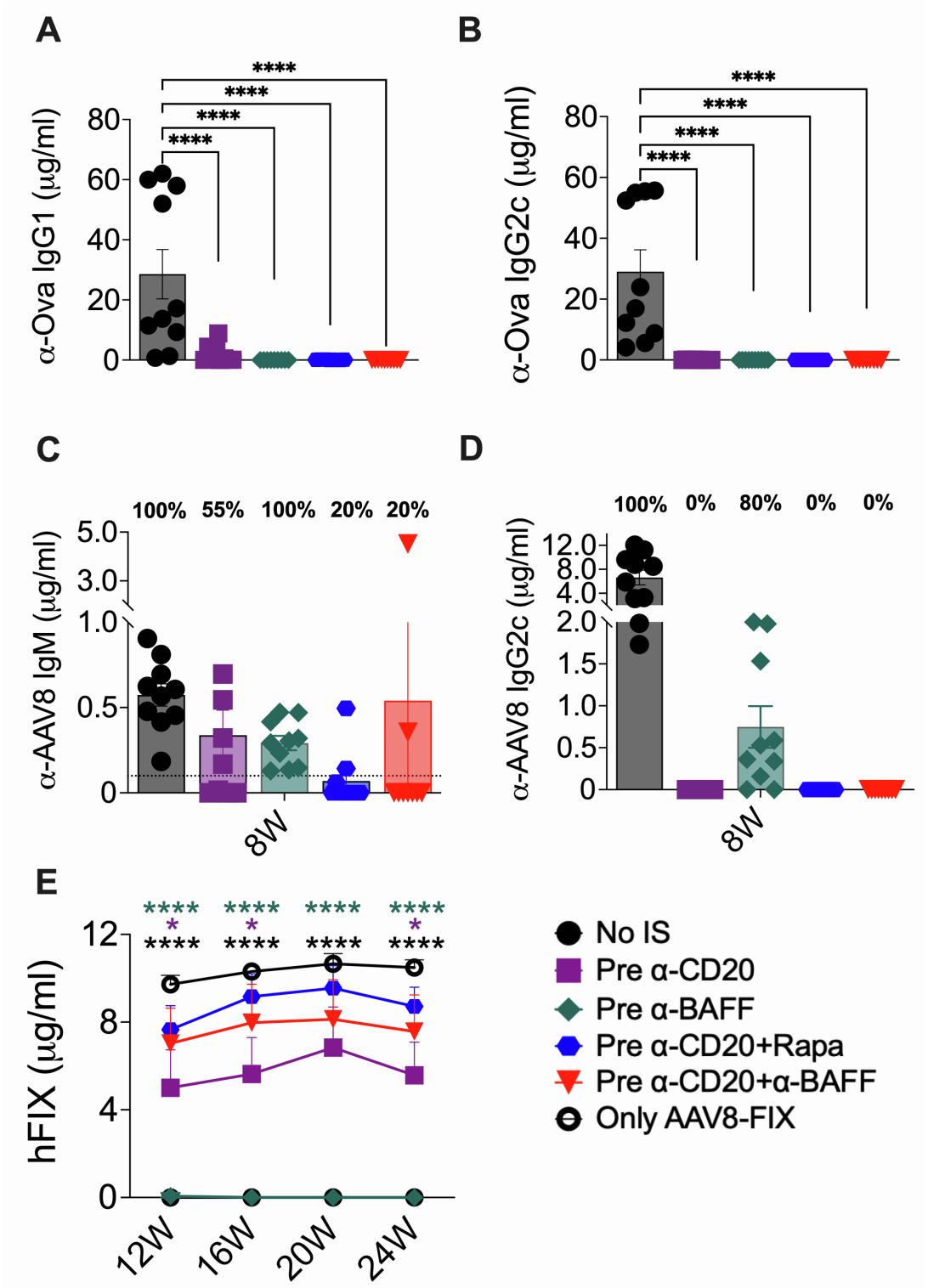

Figure S3: Effect of IS pre-treatment on primary high dose AAV administration. A and B) OVA specific IgG1 and IgG2c levels in plasma samples collected at 4 weeks post AAV8-OVA administration and IS pre-treatment (n=5-10/group). **C and D)** Frequencies of animals that develop AAV capsid specific IgM and IgG2c responses from plasma samples collected at 8 weeks post primary high dose AAV8-OVA administration. **E)** Circulating hFIX levels at different time points post re-administration with AAV8-hFIX. Statistical significance was calculated by one way ANOVA (Dunnett's multiple comparison) for **A,** and **B**, and two-way ANOVA (vs AAV8-FIX group) for **E**. *p ≤ 0.05; ****p ≤ 0.0001.

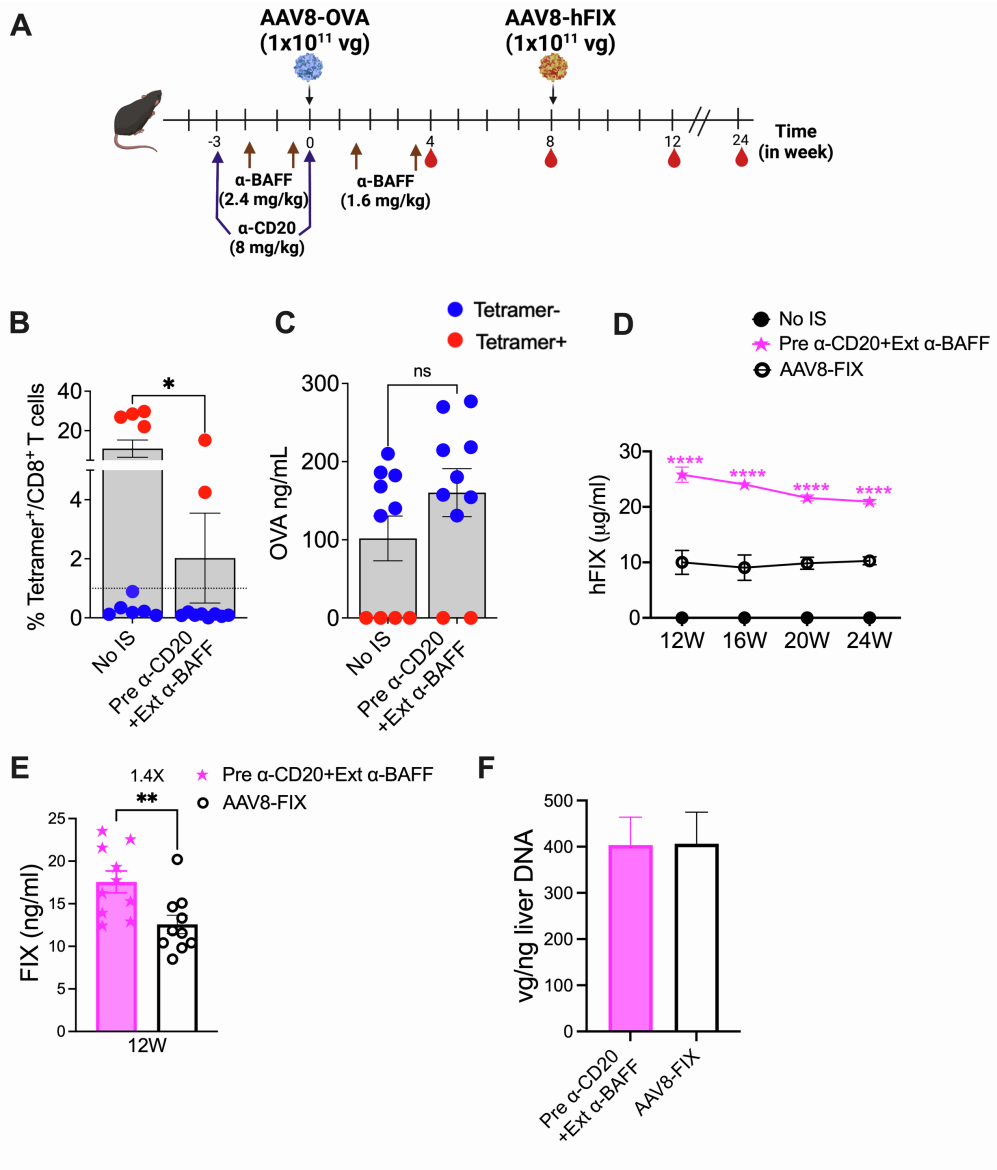

Figure S4: Effect of extending α-BAFF treatment on AAV readministration. A) Timeline **B)** CD8⁺ T cell response to OVA transgene in control (no IS) and IS pre-treatment with α-CD20 + ext α-BAFF (n=5-10/group). **C)** Circulating plasma levels of OVA at 4 weeks post AAV8-OVA administration. **D**) hFIX plasma levels at different time points post readministration. **E)** Circulating hFIX levels at 4 weeks post AAV8-hFIX re-administration. **F)** AAV vector genome (vg) copies per ng of DNA in livers of IS treated and naïve mice following AAV8-hFIX liver gene delivery. Statistical significance was calculated by one-way ANOVA (Dunnett's multiple comparisons) for **B,** and **C,** two-way ANOVA for **D,** and student's t test for **E**. *p ≤ 0.05; ****p ≤ 0.0001.

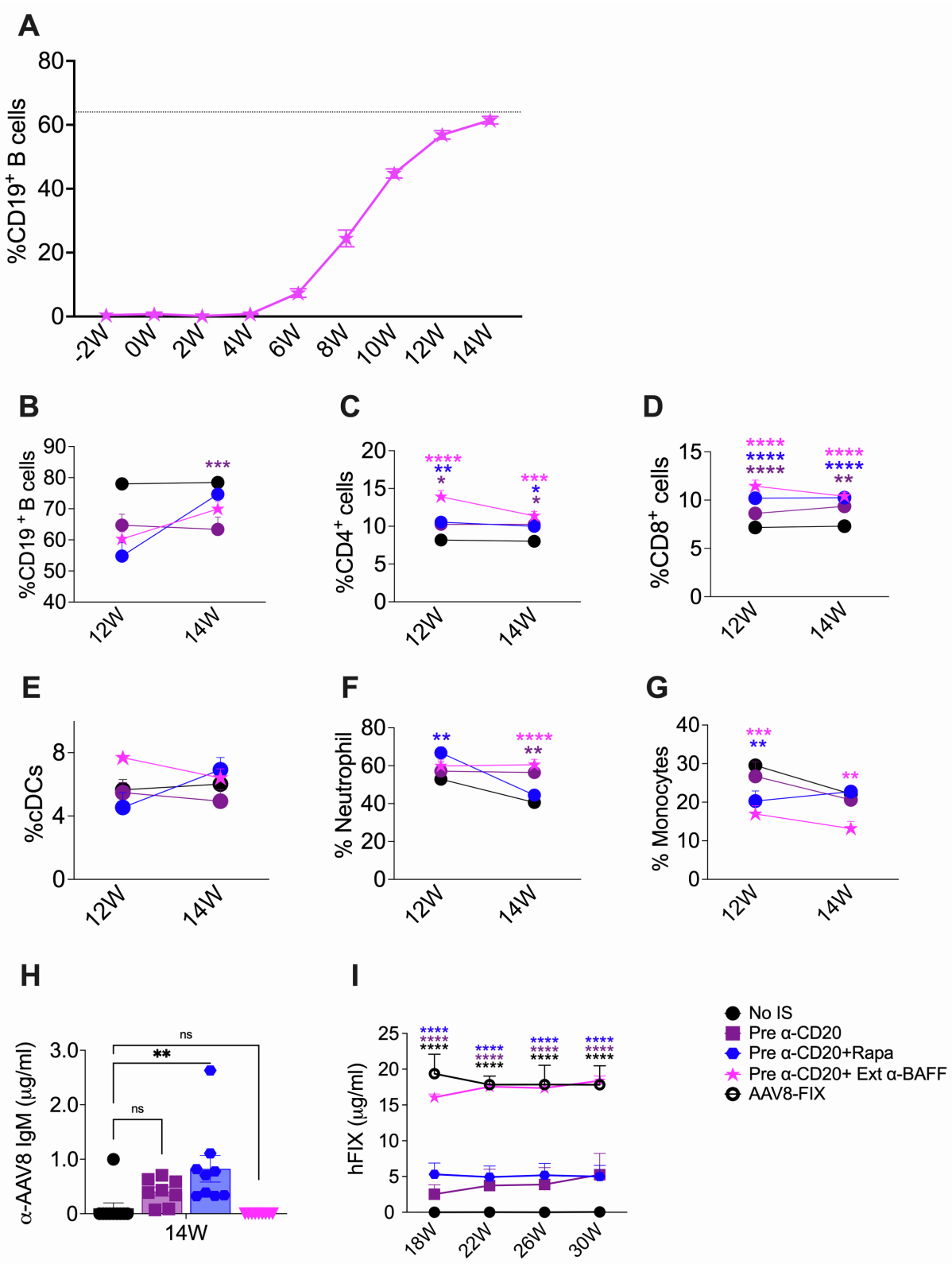

Figure S5: A) Kinetics of blood B cell repopulation following α-CD20 + ext α-BAFF treatment. Dotted line indicates % of B cells in PBMC from naïve mice. **B-G)** Repopulation of $CD19^+$ B cells, $CD4^+$, $CD8^+$ T cells, cDCs, neutrophils and monocytes at 12- and 14- weeks post IS pre-treatment (n=5-10/group). **H)** AAV8 capsid specific IgM levels at 14- weeks. **I)** Longitudinal analysis of plasma hFIX expression in control (no IS) and IS pre-treated groups re-dosed at week 15 with AAV8-hFIX. Statistical significance was calculated by one-way ANOVA (Dunnett's multiple comparisons) for **H**, and two-way ANOVA for **B-G** and **I**. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

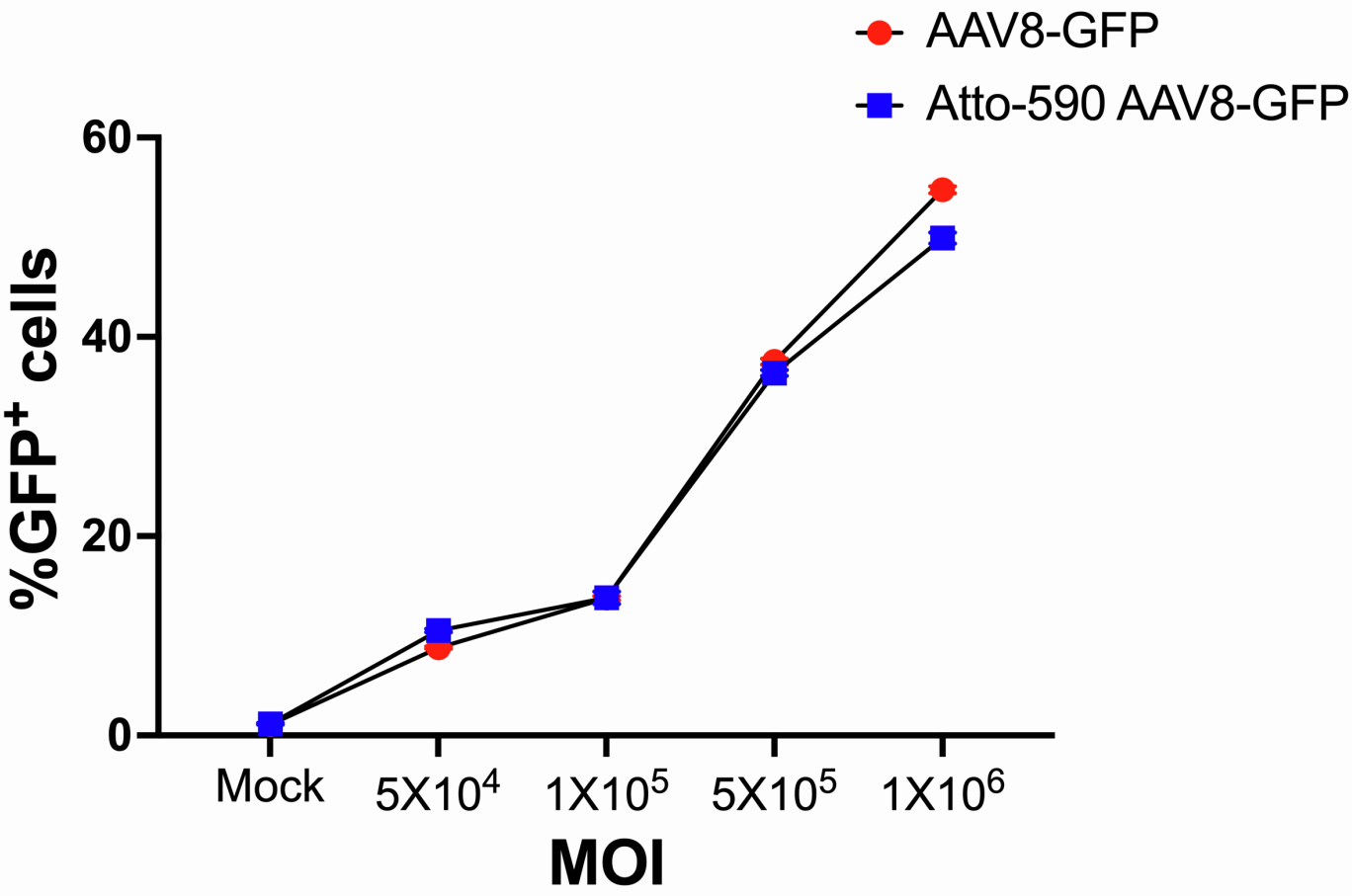

Figure S6: In vitro transduction by AAV8-GFP and Atto-590 AAV8-GFP at different MOIs in 2V6.11 cells.

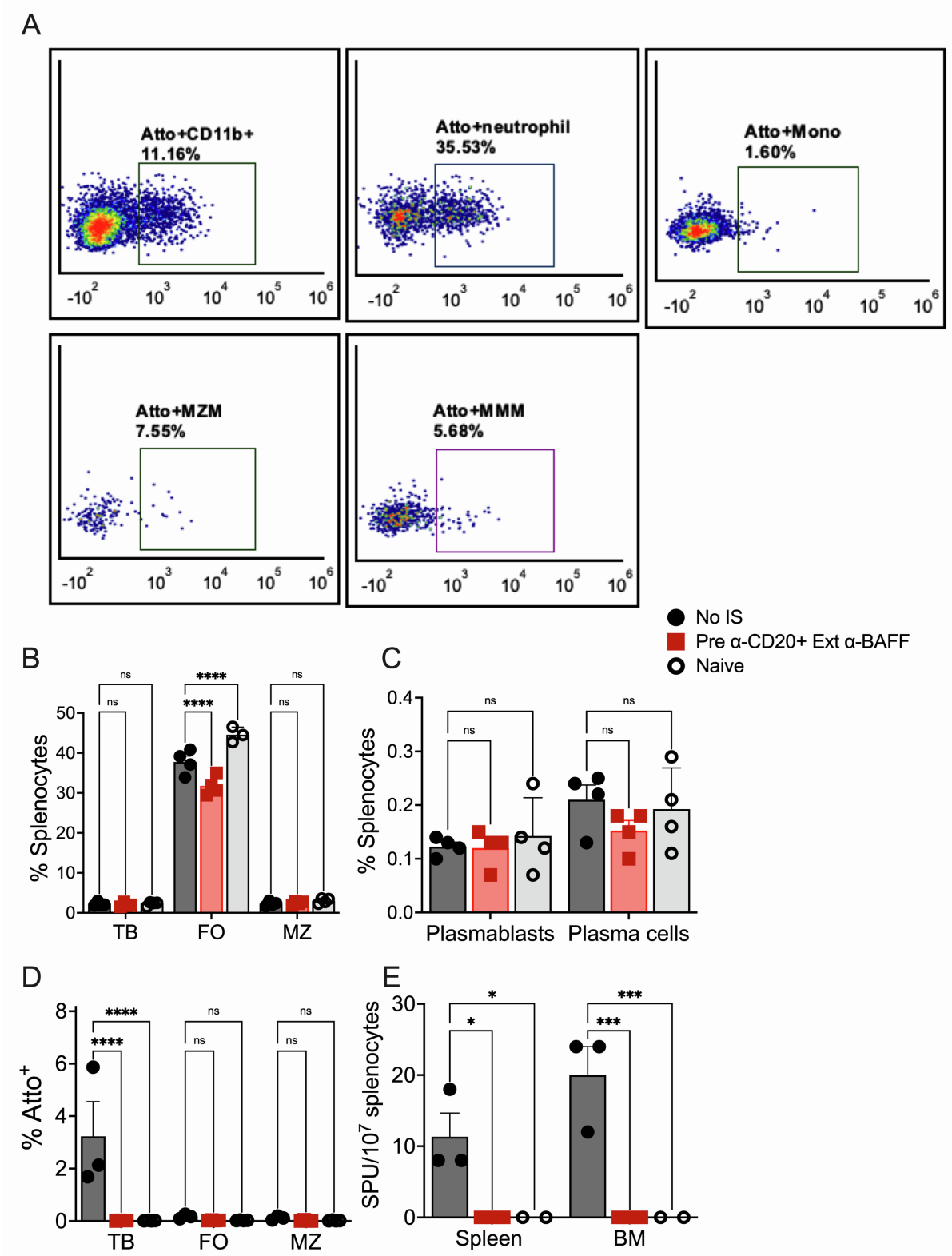

Figure S7: A) Representative density plots showing uptake of AAV8-Atto-590 in various immune populations (n=2-6/group). **B)** Frequencies of splenic transitional (TB), follicular (FO) and marginal zone (MZ) B cells from AAV8-OVA treated mice that received no IS, α-CD20+ext α-BAFF IS pretreatment, naïve animals at 12 weeks. **C)** Frequencies of plasmablasts and plasma cells at 12 weeks. **D)** AAV8-Atto-590 uptake in TB, FO and MZ B cells. **E)** AAV B cell ELISpots from spleen and bone marrow plasma cells of AAV8-OVA treated mice at 12 weeks. Statistical significance was calculated by one-way ANOVA (Dunnett's multiple comparisons) for **B-E**. *p ≤ 0.05; ***p ≤ 0.001; ****p ≤ 0.0001.